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PREPARATION OF POTENTIAL RADIOPROTECTIVE AGENTS DERIVED FROM AMINOTHIOLS

Annual Report

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1. SUMMARY

The synthesis of fifteen (15) target compounds have been completed during this year. In addition, the synthesis of several steroid derivatives which were prepared during this year are presented. During this second year we established the validity of both the "thiol + thiolsulfinate" and the "DEAD" routes to mixed disulfides of all kinds. While emphasis was placed during the early part of this year upon preparation of steroid-based potential radioprotective agents, the latter portion of this time was used to prepare other mixed disulfides.

A listing of target compounds prepared during this report period and submitted for biological evaluation follows. Their order of presentation in this summary reflects their order of presentation in SECTION IV of this report: (1) Cholestanyl Disulfide; (2) Cholesteryl Disulfide; (3) 3-Cholest-4-enyl Thioacetate; (4) 3-Mercaptocholest-4-ene; (5) 3-Stigmast-6-ene Disulfide; (6) Thioepiandrosterone; (7) Cysteaminyl 3-Thioepiandrosteronyl Disulfide; (8) Cysteaminyl 4-Hydroxyphenyl Disulfide; (9) Cysteaminyl 10-Carboxyldecyl Disulfide; (10) 2-Mercaptophenothiazine; (11) 2-Phenothiazinyl Disulfide; (12) Cystamine S-Oxide Dihydrochloride. In addition to these twelve compounds three polymer-bound substances were prepared: (13) Cysteamine bound to Dowex-50W; (14) WR-1065 bound to Dowex-50W; (15) Cystamine bound to Dowex-50W. Throughout this report the specific syntheses of target compounds begins with an italicized title.

During the third, and final year, of this contract the syntheses will focus upon mixed disulfides related to WR-2721 and to WR-3689. Particular attention will be placed upon creating mixed disulfides to small, water soluble thiols such as thioglycerol. It has been the policy with this program to explore new syntheses using mercaptoethylamine (MEA) prior to attempting syntheses with more complex molecules, specifically WR-1065. This procedure will be followed in the third year. Additionally, several steroid derivatives whose syntheses were well underway when priorities were realigned will, hopefully, be taken to a successful conclusion during this third year.

II. FOREWORD

All information in this report is the property of the U. S. Army Medical Research and Development Command. The contractor retains no copyright or patent rights.

All target compounds reported herein were prepared in strict compliance with CGMP guidelines. All final products and also compounds new to the chemical literature were characterized by both elemental and spectroscopic analyses.

Citations of commercial organizations and trade names in this report do not consititute an official Department of the Army endorsement or approval of the products or services of these organizations.

III. TABLE OF CONTENTS

IV-I. INTRODUCTION.

The purpose of this program is to prepare substances containing known (and potential) radioprotective fragments bound to small biomolecules. They are to be prepared in small (≈2 g) quantities. These compounds are then to be submitted for biological evaluation. The method chosen to link the radioprotective fragment (usually an aminothiol) to the biomolecule is to join them via a disulfide bond (-S-S-). During much of this year particular emphasis has been placed upon the synthesis of potential radioprotective steroids.

Two very different methodologies have been used for the synthesis of unsymmetric disulfides. One involves the reaction of a thiol with the thiolsulfinate derived from a different thiol. This method has the potential for producing the desired (target) compound in high purity with a minimum of side products.

$$R\text{-}S(O)\text{-}S\text{-}R + 2R'\text{-}SH \longrightarrow 2R\text{-}S\text{-}S\text{-}R' + HOH$$

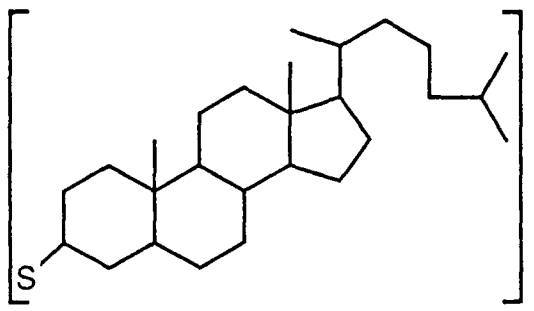
The second method involves the use of "**DEAD**" (**di**ethyl **a**zo**d**icarboxylate) to condense, sequentially, two different thiols.

$$R\text{-}SH + EtOC(O)N{=}NC(O)OEt \longrightarrow RSN(C(O)OEt)\text{-}NHC(O)OEt$$
(**DEAD**)

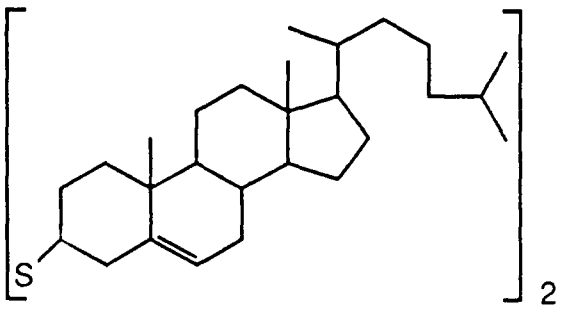
$$RSN(C(O)OEt)\text{-}NHC(O)OEt + RS'\text{-}H \longrightarrow R\text{-}S\text{-}S\text{-}R' + EtOC(O)NHNHC(O)OEt$$

Taken together, these two methods appear to provide the bulk of the synthetic methodology needed to prepare the unsymmetric disulfides now envisioned for this study.

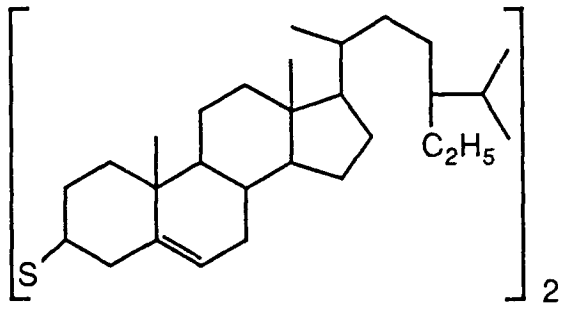
During the reporting period fifteen (15) compounds were submitted for biological evaluation. Their structures are included in Table 1. Seven of these are steroid-containing substances, two are phenothiazines and three are polymer-bound materials. The latter contain a radioprotective agent (*e.g.*, WR-1065) bound to a polymer by an ionic bond. The polymer which was used was the commercially-available Dowex 50W-X8, an ion-exchange resin containing free sulfonic acid residues.



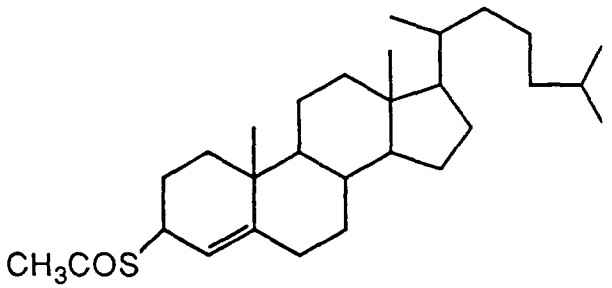
cholestanyl disulfide



cholesteryl disulfide



3-stigmast-6-ene disulfide



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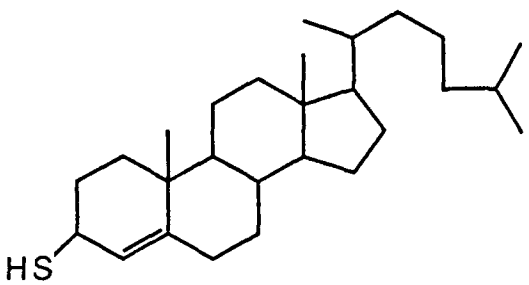
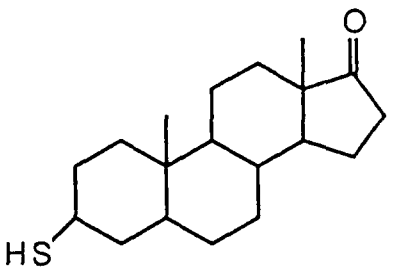
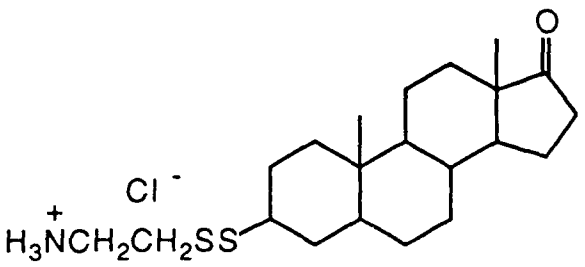


3-mercaptocholest-4-ene

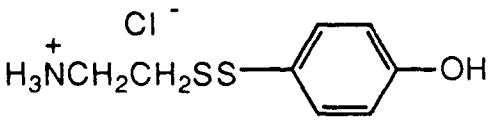


thioepiandrosterone

Table 1. Compounds Submitted This Year



cysteaminyl 3-thioepiandrosteronyl disulfide



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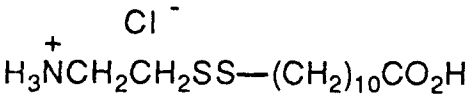
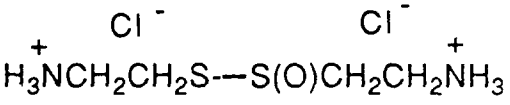
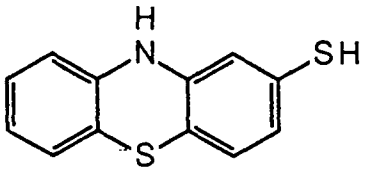
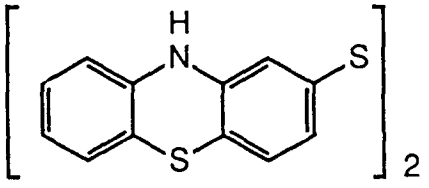


cysteaminyl 10-carboxyldecyl disulfide hydrochloride





2-mercaptophenothiazine



2-phenothiazinyl disulfide

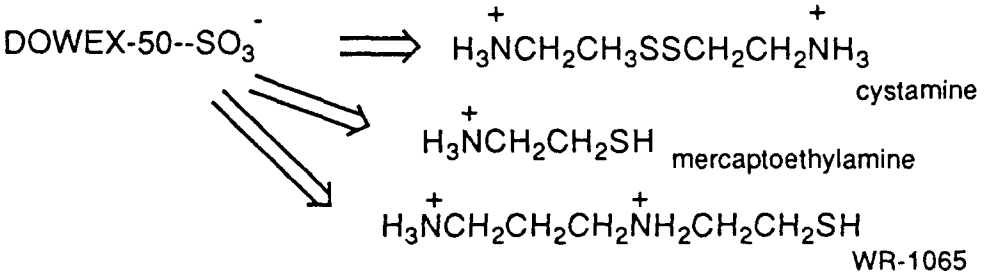
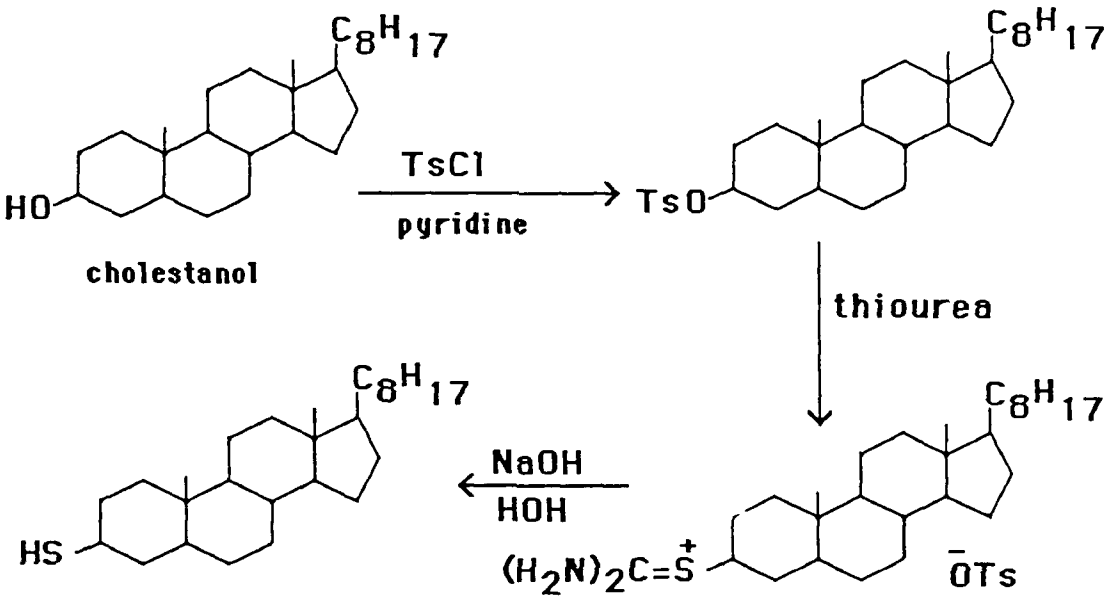


Table 1. (continued)

IV-2. SYNTHESIS OF STEROID DERIVATIVES SUBMITTED FOR ASSAY

A. CHOLESTANYL DISULFIDE

The sequence below presents the synthesis of thiocholestanol. It, in turn, may be oxidized by either oxygen or molecular iodine to the corresponding symmetric disulfide. While both oxidations have been conducted successfully in our laboratory, only the iodine approach is presented below.



1. Cholestanyl Tosylate

A mixture of cholestanol (19.5 g, 0.0500 mol), tosyl chloride (16.3 g, 0.0870 mol) and 72 mL of pyridine were placed in a 250 mL, three-necked flask equipped with a condenser, overhead stirrer and drying tube. The reaction mixture was stirred at room temperature for 22 h (some precipitate had formed after 1 h) and then poured on to ≈100 mL of ice-water. The resulting white solid was removed by filtration, washed with water and then air dried. There resulted 26.7 g (0.0492 mol, 99% yield) of the desired product, mp 131-134 °C [lit[2] mp 136 °C]

Recrystallization from a 1:1 (v/v) mixture of benzene:cyclohexane yielded 18.6 g of product, mp 134-136 °C and a second crop of 3.3 g with a mp of 132-134 °C. Though slightly different in mp, these samples had identical IR spectra.

This reaction was repeated to afford crude tosylate in >90% yield.

2. Cholestanylisothiouronium Tosylate

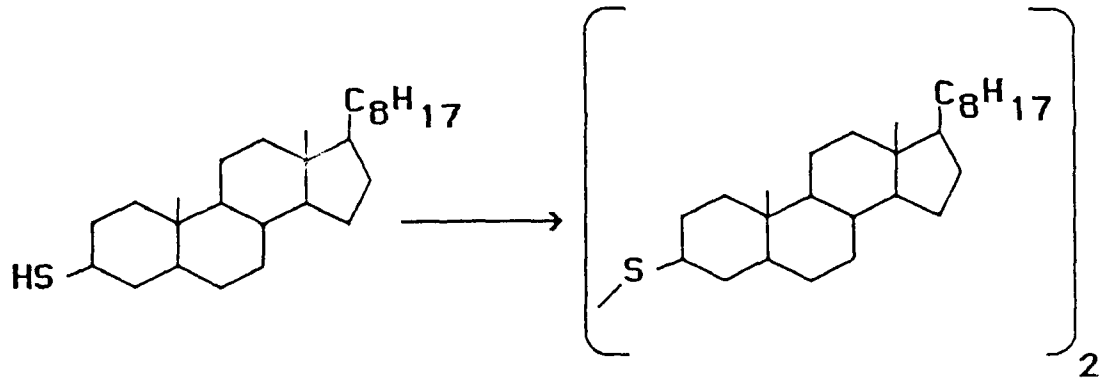
A mixture of 21.95 g (0.0400 mol) of cholestanyl tosylate (described above), 36.6 g (0.480 mol) of thiourea, 20.4 mL of pyridine and 204 mL of absolute ethanol was refluxed for 3 h in a 500 mL flask equipped with a condenser and drying tube. (The reaction mixture became homogeneous after 0.5 h.) The reaction mixture, upon cooling to room temperature, was poured on to ≈300 mL of ice water and the resulting solid removed by filtration. This solid then was suspended in 100 mL of acetone, the suspension heated to boiling, and the insoluble materials removed by filtration from the hot solution. There resulted 18.06 g (0.2920 mol, 73% yield) of crude product, mp 250-258 °C. This product was recrystallized from ethanol (≈275 mL) to yield 10.1 g (0.0163 mol, 40.8 % yield) of the desired salt, mp 270-271 °C [lit1 mp 270 °C]. An additional 1.68 g (0.00272 mol, 6.79% yield) was recovered from the second crop.

ANAL. Calcd for $C_{35}H_{58}N_2O_3S_2$: C, 67.91 %; H, 9.45 %; N, 4.53 %; S, 10.36 %
Found: C, 68.21 %; H, 9.39 %; N, 4.57 %; S, 10.61 %

3. Thiocholestanol ("Dihydrothiocholesterol")

A suspension of 9.6 g (0.016 mol) of cholestanylisothiouronium tosylate (described above) and 2.65 g (0.0662 mol) of sodium hydroxide in 195 mL of absolute ethanol was refluxed until the mixture became homogeneous. At this point 19.5 g of water was added and the reaction mixture refluxed for 2 h. Upon cooling, the reaction mixture was poured onto 300 mL of ice water and the resulting suspension acidified with 10 mL of glacial acetic acid. The desired, crude product was removed by filtration to afford 6.07 g (0.0150 mol, 99 % yield) of solid, mp 74-86 °C. This was recrystallized from ethanol to afford 3.93 g of thiocholestanol, mp 80-81 °C [lit1 mp 80-81 °C]. A second crop of 0.85 g was obtained from the mother liquor bringing the final yield to 4.78 g (0.0118 mol, 76.2 %).

4. *Cholestanyl Disulfide*



A 250 mL three-necked flask was equipped with an addition funnel, reflux condenser and magnetic stirrer. A 2.45 g (0.00599 mol) sample of thiocholestanol was placed in the flask along with 125 mL of ethanol. A 100 mL ethanol solution containing 1.0 g (0.0039 mol) of iodine was placed in the addition funnel. The mercaptan solution was heated to reflux and the iodine solution added dropwise until the solution remained colored. This required the addition of ≈ 85 mL of the I_2 solution. (The consumption of mercaptan was followed by the use of TLC .[3]) The solution was cooled in an ice bath. The product disulfide crystallized during cooling. This product was removed by filtration and washed with 50 mL of ice-cold ethanol to yield 2.12 g (85 % yield) of a white solid mp 177-177.5 °C (lit[4] mp 178-180 °C).

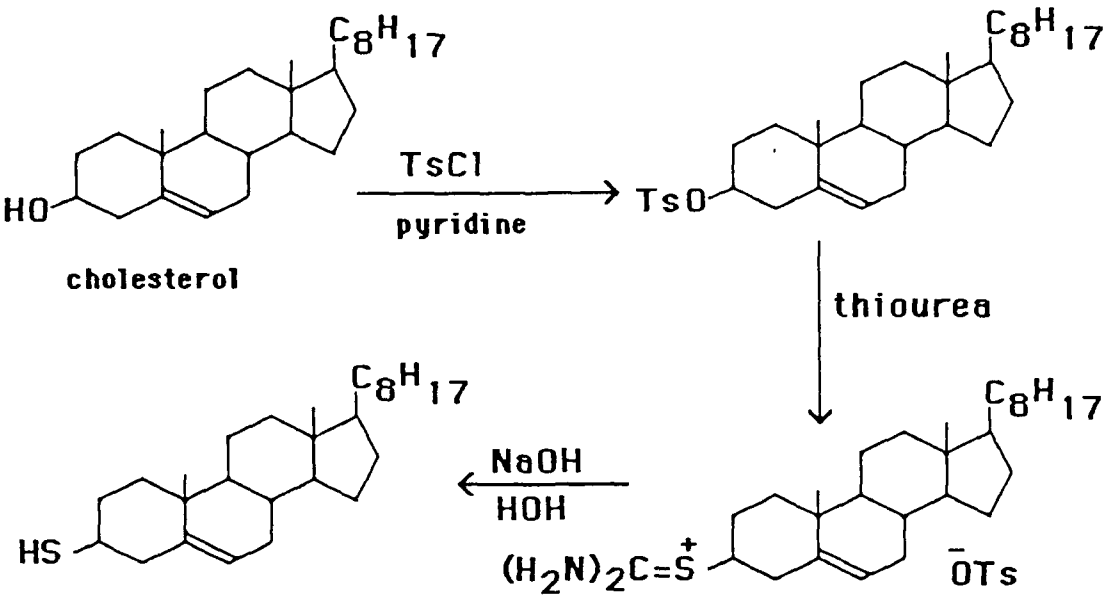
The HPLC [5] showed this product to be homogeneous. The ^1H NMR spectrum showed a complex group from 2.1 to 0.6 ppm and a small multiplet at approximately 3.2 ppm. The ^{13}C showed the presence of 27 carbons with one peak representing two carbons.

ANAL. Calcd for $C_{54}H_{94}S_2$: C, 80.32%; H, 11.74%; S, 7.94%

Found: C, 80.05%; H, 11.54%; S, 8.25%

B. CHOLESTERYL DISULFIDE

The synthesis of this compound requires four steps. The first three are shown below. The final step involves oxidation of thiocholesterol to the disulfide. This oxidation can be accomplished using several reagents, including oxygen or molecular iodine. The overall process is similar to that used to prepare cholestanyl disulfide.



1. Cholesteryl Tosylate

A mixture of cholesterol (19.3 g, 0.0500 mol), tosyl chloride (16.3 g, 0.0855 mol) and 75 mL of pyridine (dried over KOH) were stirred at room temperature for 24 h. The reaction was conducted in a three-necked flask equipped with a drying tube. The reaction mixture then was poured into ≈100 mL of ice water. The resulting precipitate was washed with 4 x 100 mL of ice water and air dried. There resulted 27.1 g (100% yield) of crude product, mp 121-126 °C. TLC (silica gel plate, chloroform eluent, iodine visualization) indicated the presence of only one substance. This dried solid was dissolved in ≈20 mL of refluxing benzene and ≈40 mL of pentane then was added; a solid precipitated. After cooling, this solid was removed by filtration to yield 20.9 g (0.0387 mol, 77% yield) of desired product, mp 127-130 °C (lit[6] mp 131 °C). This product had IR (Nujol mull), ^{1}H NMR and ^{13}C NMR spectra consistent with the assigned structure.

Two additional runs afforded the product in 51% and 82% yield.

2. Cholesterylisothiouronium Tosylate

A mixture of cholesteryl tosylate (5.4 g, 0.010 mol), thiourea (9.0 g, 0.12 mol), pyridine (5 mL) and 50 mL of absolute ethanol was refluxed for 3 h. The hot solution then was treated with sufficient water to bring it to the cloud point. The reaction mixture then was cooled to room temperature and the resulting

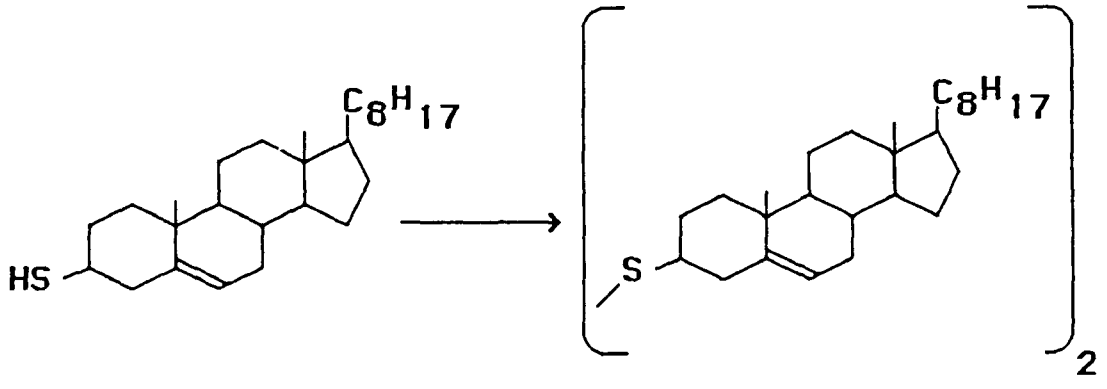
solid removed by filtration. This solid was suspended in 230 mL of acetone and the mixture heated to reflux. The insoluble salt was removed from this solution by filtration to afford 3.6 g of crude salt, mp 236-238 °C . This salt was recrystallized from ethanol to afford 2.85 g (0.00462 mol, 46 % yield) of salt, mp 237-239 °C (lit[7] mp 230-234 °C).

ANAL. Calcd for $C_{35}H_{56}N_2O_3S_2$: C, 68.10 %; H, 9.15 %; N, 4.54 %; S, 10.40 %
Found: C, 68.20 %; H, 9.39 %; N, 4.57 %; S, 10.60 %

3. Thiocholesterol

A mixture of cholesterylisothiouronium tosylate (2.46 g, 0.00400 mol), sodium hydroxide (0.068 g, 0.012 mol) and 50 mL of absolute ethanol was refluxed until the system became homogeneous (about 0.5 h). After this, 5 mL of water was added and the mixture heated for an additional 2 h. The reaction mixture then was poured on to 100 mL of ice water and treated with ≈1 mL of glacial acetic acid. The resulting, slightly acidic mixture was filtered and the resulting solid recrystallized from 5:1 acetone:methanol. There resulted 1.2 g (0.0030 mol, 75% yield) of thiocholesterol, mp 90-92 °C (lit[6] mp 94-96 °C). The TLC[8] of this substance indicated a single spot. The IR spectrum and the ^1H and ^{13}C NMR spectra were consistent with the assigned structure.

4. *Cholesteryl Disulfide*



A 5.67 g (0.0141 mol) sample of thiocholesterol was placed in a 500 mL erlenmeyer flask and to this was added 100 ml of hexane. The resulting solution was stirred and to this was added 100 mL of an aqueous 5 % KOH solution containing 3.62 g (0.0130 mol) of iodine. The bilayer system was stirred vigorously until the iodine color had disappeared (≈ 40 min). After this time, the mixture was diluted with 200 mL of water and then extracted with 3 x 100 mL of hexane. The hexane extracts were collected and dried (anhydrous magnesium sulfate). The solvent was removed by rotary evaporation at reduced pressure to

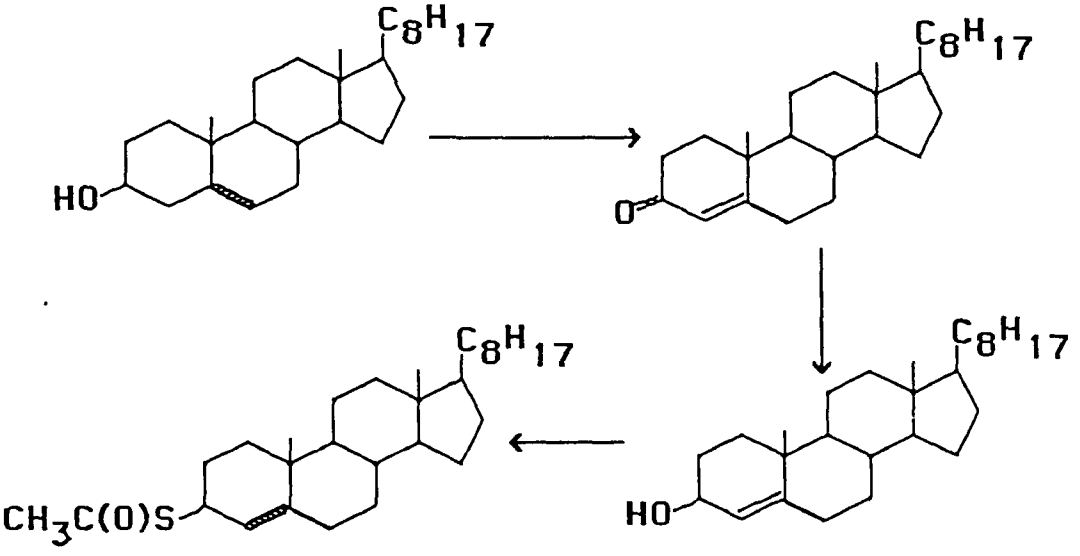
yield 5.28 g of a light yellow solid, mp 130-140 °C. This solid was recrystallized by dissolving it in a minimal amount of hot benzene and adding acetone until the cloud point was reached. The pure solid crystallized upon cooling. The solid was filtered and washed with 100 mL of ice cold ethanol. This yielded 4.2 g (0.0053 mol) of the desired compound, mp. 141-142.5 °C (lit mp 141-143 °C).

The HPLC [9] of this solid showed the presence of only one component. The ^1H NMR spectrum showed a complex group from 2.7 to 0.5 ppm and a doublet at 5.36 ppm. The ^{13}C spectrum showed 25 carbon resonances with three peaks possibly containing two carbons.

ANAL. Calcd for $C_{54}H_{90}S_2$: C, 80.73%; H, 11.29%; S, 7.98%
Found: C, 80.43%, H, 10.88%; S, 7.77%.

C. 3-CHOLEST-4-ENYL THIOACETATE

The sequence below employs an Oppenauer oxidation of cholesterol to isomerize the double bond into the A ring of the steroid. Following this, a two step sequence is used to convert the keto group at C3 to a thioacetoxyl group ($CH_3C(O)S$-).



1. Cholest-4-en-3-one

Cholesterol (40 g, 0.103 mol), dried acetone (270 mL), benzene (300 mL) were placed in a 2L three necked flask which was protected from the

atmosphere with a drying tube. The mixture then was heated to reflux. To the hot solution there was added, in one portion, a solution of 32 g of aluminum t-butoxide in 220 mL of benzene. Within 15 min the solution became cloudy and turned yellow. The reaction mixture then was refluxed for 1 h. Upon cooling, the mixture was diluted with 80 mL of water and 200 mL of 10% sulfuric acid. The resulting mixture was treated with 600 mL of water and then extracted with three 50 mL portions of benzene. The combined benzene layers were washed with water, dried (magnesium sulfate) and the solvent removed under reduced pressure (rotary evaporator). The residual yellow oil was dissolved in 30 mL of hot acetone and the acetone solution diluted with 100 mL of hot methanol. A white solid, which deposited upon cooling and scratching, was removed by filtration. There resulted 18.15 g of crude product, mp 77-78 °C. The volume of the filtrate was reduced in half to yield a second crop of 16.6 g , mp 60-66 °C.

This procedure was repeated two additional times to yield a total of 66.44 g of crude product. This was dissolved in 75 mL of refluxing acetone and 100 mL of methanol. The solution was filtered while still hot and allowed to cool slowly to yield 57.37 g of the desired product, mp 80-81 °C (lit[10] mp 79-80 °C). The average yield for these procedures was 74 %.

2. Cholest-4-en-3-ol

4-Cholesten-3-one (28 g, 0.073 mol) was dissolved in 200 mL of ethanol and added, dropwise, to a solution of 4 g (0.1 mol) of sodium borohydride in 40 mL of water at 35 °C. By the time addition was complete a white precipitate had formed. The mixture was stirred for an additional 20 min and then was treated with 60 mL of water and refluxed for 10 min to destroy excess reducing agent. The reaction mixture, upon cooling, was poured on to 500 mL of ice-water and extracted with three 250 mL portions of ether. The ethereal solution was washed with water (to pH ≈7), dried (magnesium sulfate), the drying agent removed, and the ether evaporated to yield 28.41 g (100 %) of crude product, mp 105-110 °C. This was recrystallized from ethanol to yield 19.72 g of product, mp 121-123 °C. Concentration of the mother liquor to half of the original volume afforded 3.8 g of a second crop, mp 120-122 °C.

This reaction was repeated two additional times to provide yields of 83, 86 and 81 %.

3. *3-Cholest-4-enyl Thioacetate*

Diisopropyl azodicarboxylate (12.51 g, 0.06190 mol) was added to a stirred solution of 15.75 g (0.06000 mol) of triphenylphosphine dissolved in 150 mL of tetrahydrofuran at ice-bath temperatures. The mixture was stirred for 0.5 h during which time a white precipitate formed. To this there then was added, dropwise and with stirring, a solution of 4-cholesten-3-ol (11.64 g, 0.03026 mol) and thioacetic acid (4.54 g, 0.0596 mol) dissolved in 75 mL of tetrahydrofuran. After stirring for one hour at ice-bath temperatures, the reaction mixture was stirred at room temperature for an additional hour. The solvent was removed from the resulting clear yellow solution under reduced pressure. To the resulting

mixture there was added ≈ 250 mL of hexane. The resulting mixture was refluxed for 15 min and then filtered hot. The process was repeated until no further material dissolved in the hexane. The hexane extracts were combined and the solvent removed using a rotary evaporator. The resulting solid was recrystallized from ethanol to afford 6.43 g (0.0145 mol; 48 % yield) of the desired product, mp 110-111 °C.[11]

This process was repeated four additional times. Yields varied from 32.5 to 44.0 % for these additional syntheses. Melting points varied from 110-113 °C over this entire set of compounds.

ANAL. Calcd for $C_{29}H_{48}OS$: C, 78.31%; H, 10.88 %; S, 7.21 %
Found: C, 77.91%; H, 10.91 %; S, 7.49 %

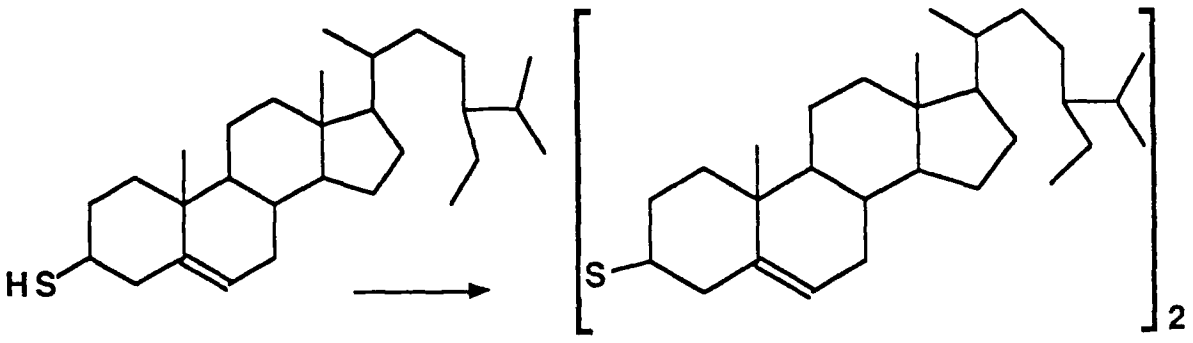
D. *3-MERCAPTOCHOLEST-4-ENE*

The thioester described above (4.4 g, 0.01 mol) was dissolved in 20 mL of ether. This solution then was added, dropwise and with stirring, to a suspension of 0.8 g (21 mmol) of lithium aluminum hydride in 20 mL of ether. This mixture was stirred at room temperature for 30 min and the excess reductant destroyed by the careful addition of ≈ 12 mL of 1N hydrochloric acid. The ether layer was separated, washed with water (to pH ≈7), and then dried with magnesium sulfate. Evaporation of the ether afforded 3.76 g (99% yield) of crude product, mp 85-87 °C. Recrystallization from ethanol under a nitrogen blanket afforded 3.0 g (76 % yield) of the desired thiol, mp 88-89 °C.

The reaction, as described above, was repeated to afford an additional 2.68 g of pure thiol. This compound had an IR spectrum, and ^1H and ^{13}C NMR spectra consistent with the assigned structure.

ANAL. Calcd for $C_{27}H_{46}S$: C, 80.52 %; H, 11.51 %; S, 7.96 %
Found: C, 80.35 %; H, 11.25 %; S, 7.66 %

E. *3-STIGMAST-6-ENE DISULFIDE (ß-SITOSTERYL DISULFIDE)*

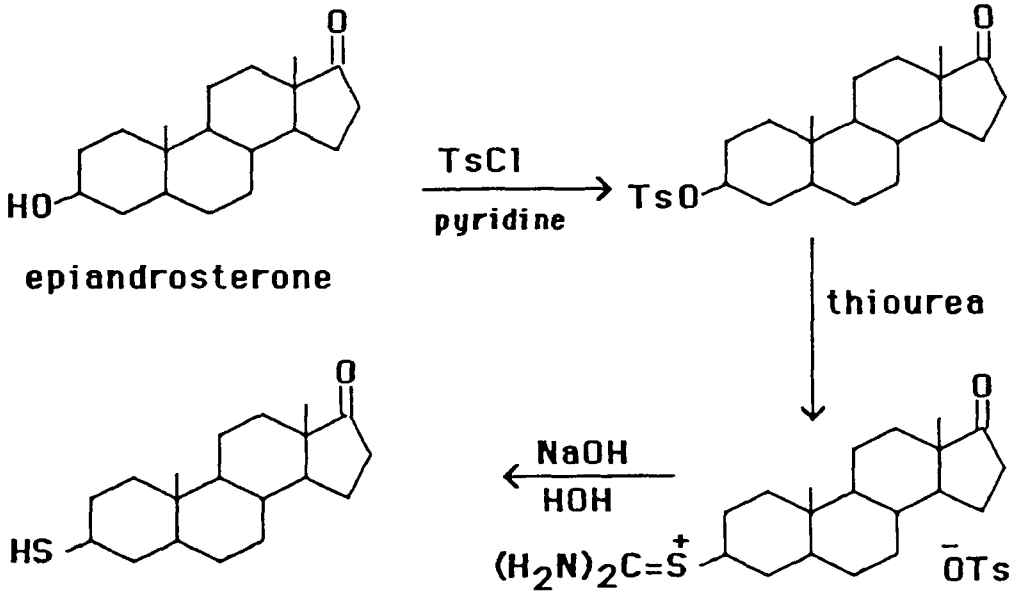


A sample of ß-thiositosterol (8.65 g, 0.0200 mol) was dissolved in 250 mL of ethanol. To this there was added, dropwise and with stirring, a solution of iodine dissolved in ethanol. Addition was continued until the color of the iodine persisted for more than 10 seconds. To this solution there was added 500 mL of ice-cold water and the resulting precipitate removed by filtration. The solid was washed with 15 mL of cold ethanol and then air-dried. There resulted 8.2 g of an off-white solid, mp 185-189 °C, which appeared to be homogeneous on TLC (8" silica gel, chloroform eluent, iodine visualization. This was recrystallized from ethanol to yield 6.5 g (75.1% yield) of product, mp 189-190 °C.

ANAL. Calcd for $C_{28}H_{98}S_2$: C, 81.05%; H, 11.49%; S, 7.46%
Found: C, 80.79%; H, 11.23%; S, 7.20%

F. THIOEPIANDROSTERONE

All of the steroid derivatives described above have lacked a functional group on the "D" ring. In this synthesis, however, there is a carbonyl group on this ring. As with the other systems, the synthesis involves an isothiouronium salt as an intermediate.



1. Epiandrosteronyl Tosylate

A mixture of epiandrosterone (13.36 g, 0.04600 mol), tosyl chloride (11.24 g, 0.05896 mol) and 50 mL of anhydrous pyridine was stirred at room temperature for 17 h. During this time the reaction mixture was protected from moisture with a drying tube. The reaction mixture then was poured into 200 mL of ice water. The resulting white precipitate was removed by filtration, washed with cold water, and then air-dried overnight. The result was 20.39 g of crude product, mp 154-158 °C. The product was dissolved in ≈ 50 mL of benzene and ≈ 60 mL of pentane then was added to the solution. A white precipitate resulted. The product, mp 156-158 °C, weighed 15.45 g (0.03475 mol, 75.55 % yield). This product had IR, ^{1}H, and ^{13}C nmr spectra consistent with the assigned structure.

Two further syntheses afforded the crude product in 96 and 92% yields.

2. Epiandrosteronylisothiouronium Tosylate.

A mixture of 12.2 g (0.0274 mol) of the tosylate described above, 27.7 g (0.364 mol) of thiourea and 277 mL of absolute ethanol was refluxed for 3 h. The reaction was carried out in a flask protected from moisture with a drying tube. The resulting white solid was removed by filtration, air-dried overnight, suspended in 300 mL of acetone and the mixture refluxed for 10 min. The insoluble material was removed from the hot solution by filtration and dried to yield 6.92 g of solid, mp 280-282 °C (lit mp 286 °C (dec)). The mother liquor was reduced in volume by 1/3 to yield an additional 0.74 g of solid, mp 276-278 °C. These two fractions were combined and recrystallized from ethanol to afforded 7.01 g [4.64 g in first crop and 2.37 g in second] of salt, mp 282-283 °C. These crops had IR, ^{1}H and ^{13}C NMR spectra consistent with the assigned structure.

ANAL. Calcd for $C_{27}H_{40}O_4S_2N_2$: C, 62.27 %; H, 7.74 %; N, 5.38 %; S, 12.31 %
Found: C, 62.04 %; H, 7.73 %; N, 5.41 %; S, 12.57 %.

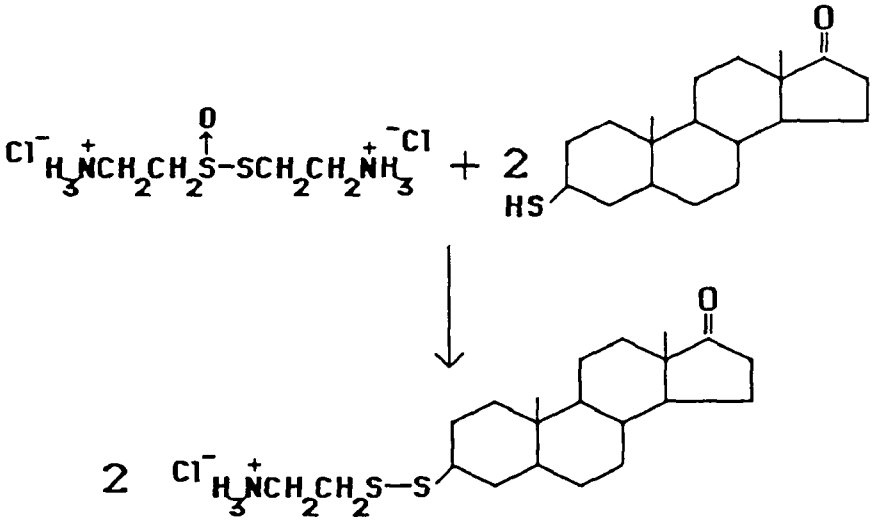
3. Thioepiandrosterone

A suspension of 0.77g of the salt described above and 0.25 g of sodium hydroxide, in 18 mL of anhydrous ethanol, was refluxed for 0.5 h.[12] The mixture was poured into 50 mL of ice water and the resulting suspension acidified (pH ≈5) with acetic acid to afford a white solid. The solid was removed by filtration, washed with water and and air dried to give 0.44 g (95 %) of the crude product, mp 133-136 °C. The product was dissolved in refluxing hexane and a small amount of insoluble material removed by filtration. The hexane was removed under reduced pressure to afford 0.40 g (86 %) of a white solid , mp 137-138 °C (lit[1] mp 138-139 °C).

ANAL. Calcd for $C_{19}H_{30}OS$: C, 74.45%; H, 9.87%; S, 10.45%
Found: C, 74.42%; H, 9.61%; S, 10.61%

G. *CYSTEAMINYL 3-THIOEPIANDROSTERONYL DISULFIDE*

This synthesis illustrates the application of the thiolsulfinate derived from cystamine to the preparation of a steroidal mixed disulfide. (The change in emphasis halted our efforts to prepare the corresponding analog of WR-1065.)



A 500 mL three necked flask was equipped with an overhead stirrer, addition funnel and drying tube. Into the flask there was placed 2.0 g (0.0083 mol) of cystamine S-oxide and 150 mL of a 1:1 (v/v) chloroform/methanol solution. A solution of 50 mL of chloroform containing 3.95 g (0.0129 mol) of 3-thioepiandrosterone was placed in the addition funnel. This solution was added, dropwise, over a period of 1 h and the resulting suspension stirred at room temperature for four days.[13] The reaction mixture then was filtered and the solvent removed from the filtrate (rotary evaporator) to yield a white solid. This solid was dried (mechanical vacuum pump) for 5 h to afford 5.3 g of a white solid, mp 198-204 °C. Recrystallization from ethanol yielded 3.61 g of material, mp. 208-210 °C. This material was recrystallized from ethanol to yield 3.28 g of the desired compound, mp 209.5-210 °C. The HPLC [14] failed to show the presence of a second component.

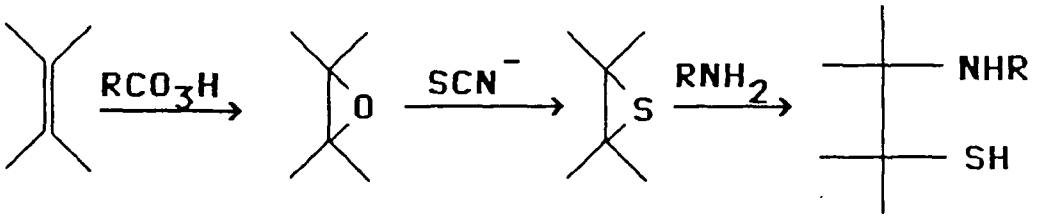
ANAL. Calcd for $C_{21}H_{36}NOS_2Cl$: C, 60.32%; H,8.36%; N,3.35%; S,15.34%
Found: C, 60.35 %; H,8.56%; N,3.50%; S,15.59%

IV-3. SYNTHESIS OF STEROID DERIVATIVES WHICH WERE NOT SUBMITTED FOR ASSAY

A changing emphasis relegated the preparation of *steroid-based* radioprotectives to an inferior position. Consequently, some synthetic efforts being conducted with steroids have not led to "target" compounds but have, instead, been placed on "hold". One example of this is a scheme designed to

create aminothiols where both the amino and the mercapto groups are part of a steroid nucleus. Specifically, the objective was to ultimately place mercapto and amino groups on adjacent carbons in the A ring of a steroid. (When this was completed the plan called for a similar preparation involving the D ring).

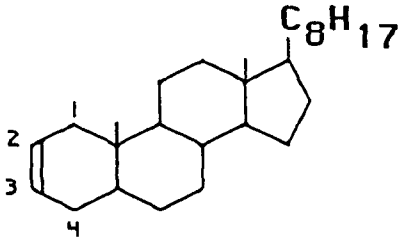
While several synthetic avenues were envisioned, they all had a common termination sequence, *i.e.*, the ring opening of an episulfide with some appropriate amine. Working backward, the episulfide would have been prepared from the corresponding epoxide which, in turn, would have been prepared from an alkene. This series of reactions is outlined below.



In a sequence such as this if, for example, 1,3-propanediamine were the starting amine, then the ultimate product would have been a derivative of WR-1065 where the ethano portion of WR-1065 had been incorporated into the steroid A ring.

Such a sequence requires the availability, in significant quantities and purity, of the starting alkene free of isomeric alkenes and cyclopropanes. To this end we embarked upon, and devoted a significant effort during this year, to the preparation of steroidal alkenes. The experiments on the following pages chart a few of our attempts, ultimately successful, to have available a steroidal alkene in high yield and purity. The desired compound is cholest-2-ene.

A. CHOLEST-2-ENE

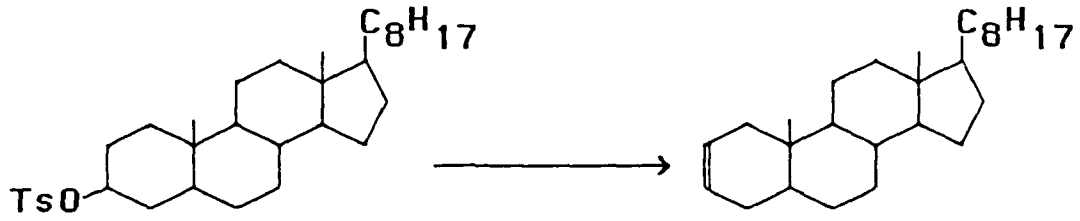


cholest-2-ene

Two routes were taken to cholest-2-ene. The first involved conversion of the corresponding alcohol to the tosylate and then elimination to given the alkene. Unfortunately, this sequence inevitably produced a mixture of 2- and 3-enes; we never were able to produce pure cholest-2-ene by this route. The second approach, and one which was successful, led to pure cholest-2-ene via

a multistep synthesis beginning with cholestanol. Some of the unsuccessful tosylate attempts are given first.

1. Attempted Preparation of Cholest-2-ene--Gamma-Collidine Route



A mixture of 6.12 g (0.0135 mol) of cholestanyl tosylate (synthesis described above) and 40 mL of 2,4,6-trimethylpyridine (gamma-collidine) was refluxed for 6 h. The collidine was removed by vacuum distillation and the residue treated with 30 mL of 10% sulfuric acid. The product was extracted with ether (2 x 50 mL) and the extracts combined. These then were washed with concentrated sodium bicarbonate solution, dried (sodium sulfate), the drying agent removed by filtration, and the solvent evaporated to afford 4.56 g of solid . The TLC of this solid[15] showed three spots. This solid was column chromatographed on Activity Grade I alumina (2.4 cm x 30 cm column) using petroleum ether (bp 30-65 °C) as the eluent to yield 1.60 g of white solid. In turn, this was recrystallized from acetone to yield 1.35 g (3.64 mmol; 32.3% yield) of needle-like crystals , mp 69-70 °C (lit[16] mp 66-68 °C, 68-69 °C) .

ANAL. Calcd for $C_{27}H_{46}$: C, 87.49%; H, 12.51%
Found: C, 87.44%; H, 12.49%

While this sample had an acceptable elemental analysis, spectroscopic evidence (particularly ^{13}C NMR) suggested that it was a mixture of cholest-2-ene and cholest-3-ene. Consequently, the reaction was repeated, as described below.

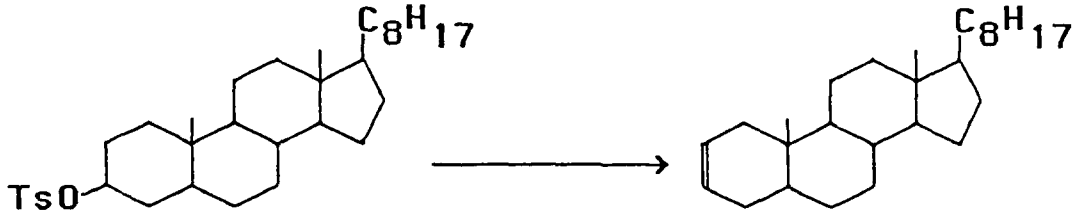
2. Attempted Preparation of Cholest-2-ene--Gamma-Collidine Route

A mixture of 22.1 g (0.0410 mol) of cholestanyl tosylate and 100 mL of gamma-collidine was refluxed for 8 h. The collidine was removed by vacuum distillation (0.17 torr; 30-32 °C). The residue was treated with 200 mL of 10% sulfuric acid and the product extracted with ethyl ether. The ether layer was washed with saturated aqueous sodium bicarbonate, dried (sodium sulfate), the drying agent removed by filtration, and the solvent removed using a rotary evaporator.

The crude product (15.71 g) was recrystallized from acetone to afford 12.55 g of a crystalline solid. An additional recrystallization from acetone yielded 10.16 g of needle-like crystals, mp 64-66 °C.

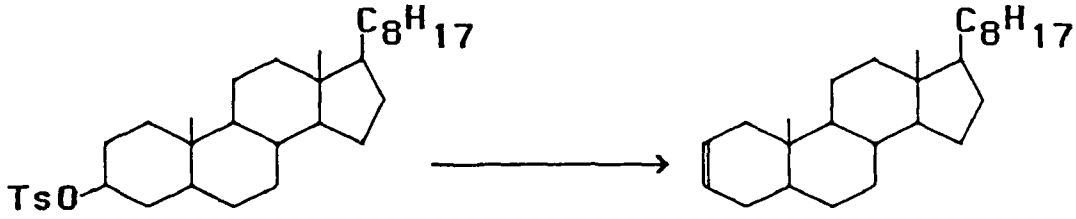
A 0.32 g sample of this solid was recrystallized from acetone to yield 0.23 g of a white solid whose ^{1}H NMR ($CDCl_3$) showed signals at 5.82-5.02 ppm (2H, m) and 2.36-0.31 ppm (57H, m). The ^{13}C NMR ($CDCl_3$) showed signals at 131.47; 125.95; 125.84; 125.41; 56.53; 56.32; 54.11; 53.44; 45.94; 42.76; 42.52; 41.49; 40.11; 40.07; 39.82; 39.54; 36.21; 35.83; 35.64; 34.91; 34.61; 34.15; 32.12; 31.86; 30.35; 28.81; 28.26; 28.02; 27.52; 24.23; 23.87; 23.535; 22.83; 22.59; 21.10; 20.95; 18.71; 12.19; 12.01; 11.89, and 11.71 ppm. These data confirmed that this sample was a mixture of cholest-2-ene and cholest-3-ene. (Chemical shifts at 131.47 ppm and 125.41 ppm corresponded to the cholest-3-ene, while those at 125.95 ppm and 125.84 ppm corresponded to the cholest-2-ene.)[17]

3. Attempted Preparation of Cholest-2-ene-- Basic Alumina Route



The starting tosylate (0.60 g) was chromatographed at -10 °C (basic alumina; methylene chloride eluent) to give 0.54 g of a white solid, mp 135-136 °C. The IR spectrum showed absorptions at 810 cm^{-1} (1,4-disubstituted benzene); 1357 cm^{-1} and 1174 cm^{-1} (-SO_2- peak); and 550 cm^{-1} (-SO_2- scissor). The "product" was starting material rather than cholest-2-ene.

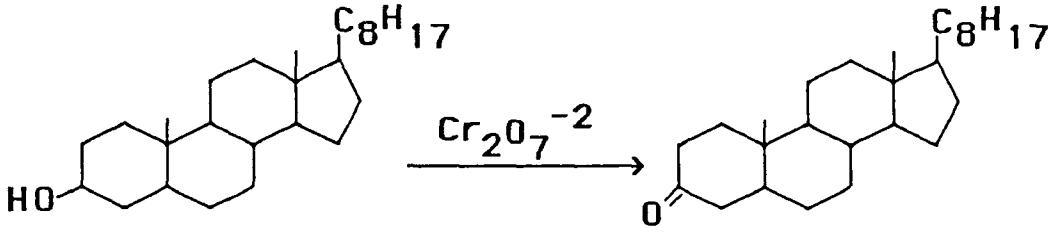
4. Attempted Preparation of Cholest-2-ene-- Resin Catalyzed Elimination



Cholestanyl tosylate (0.200 g, 0.368 mmol) was dissolved in 10 mL of methylene chloride and treated with 0.52 g (capacity = 3 meq/g) of a basic ion-exchanged resin (IRA-410). The reaction mixture was stirred at room temperature for 1 h. The TLC[18] of the resulting solution showed the presence of only starting material. The TLC results were the same after the mixture was stirred for 2 days. After two days the resin was removed by filtration and the filtrate evaporated *in vacuo* to give 0.15 g of starting material (mp 133-137 °C. (lit mp's: cholest-2-ene[19], 66-68 °C; cholestanyl tosylate[1], 136-138 °C).

Because these various eliminations failed to produce pure cholest-2-ene, a completely different scheme was attempted. This second route[20] begins with the oxidation of cholestanol to cholestanone.

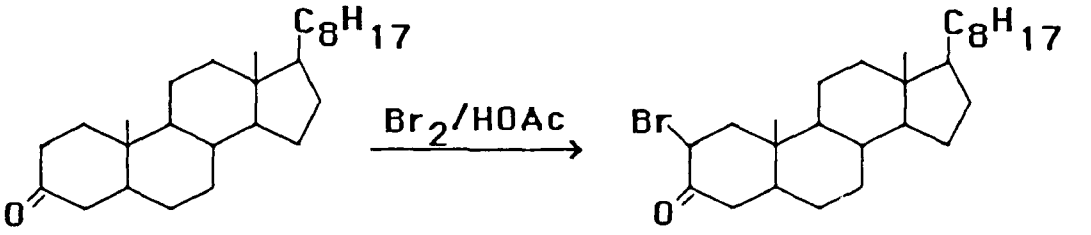
5. <u>Cholestan-3-one</u>



A hot solution of 13.9 g (0.0467 mol) of sodium dichromate dihydrate in 85 mL of acetic acid was added to a suspension of 13.4 g (0.0345 mol) of cholestanol in 75 mL of acetic acid. After warming for a few minutes to effect solution, the reaction mixture was allowed to stand overnight at room temperature. The resulting paste then was treated with 20 mL of water and the precipitate washed thoroughly with water to yield 14.4 g of a white solid, mp 124-126 °C. Its IR spectrum possessed a carbonyl absorption at 1718 cm^{-1}. This solid was recrystallized from a mixture of ethanol-acetone (4:1 v/v) to yield 8.61 g, (47.7%) of the desired compound as a white solid, mp 128-129 °C (lit[1] mp 127-128 °C).

The ^{13}C NMR spectrum ($CDCl_3$) possessed twenty-six peaks: 212.05; 56.26; 53.80; 46.70; 44.71; 42.58; 39.90; 39.49; 38.55; 38.18; 36.13; 35.76; 35.63; 35.39; 31.71; 28.96; 28.22; 27.99; 24.21; 23.81; 22.80; 22.54; 21.44; 18.65; 12.06; 11.46 ppm.

6. <u>2β-Bromocholestan-3-one</u>



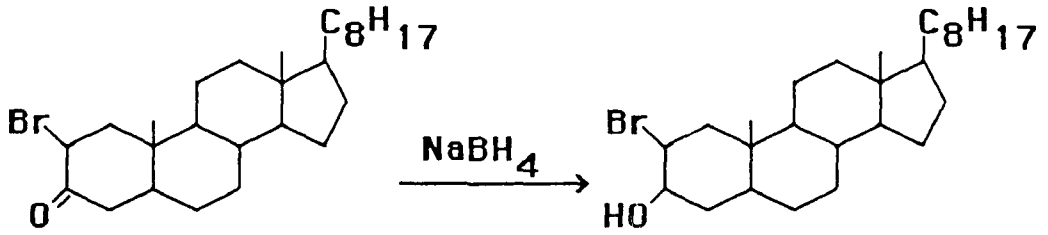
Into a three necked flask equipped with an addition funnel was placed 8.00 g (0.0207 mol) of cholestan-3-one and 350 mL of glacial acetic acid. A solution of 3.64 g (0.0228 mol) of bromine and 6 drops of 48 % hydrobromic acid in 20 mL of acetic acid was added slowly to the stirred acetic acid solution; addition required 20 min.

The resulting white precipitate (5.55 g) was removed by filtration, and the filtrate treated with 100 mL of water. There resulted 2.75 g of a white solid. The 5.55 g sample was recrystallized from a mixture of ethanol-acetone (5:1 v/v) to yield 4.44 g of a white solid, mp 160-163 °C. The 2.75 g sample was similarly recrystallized to afford 2.20 g of a white solid, mp 148-150 °C).

The two recrystallized solids were combined and recrystallized from the ethanol-acetone mixture to yield 3.21 g of a white solid, mp 165-166 °C. Reduction of the volume of the filtrate by one-half yielded a second crop (0.80 g) of product, mp 161-160 °C. The filtrate was evaporated to dryness to yield 0.72 g of solid, mp 144-147 °C (lit[19] mp 168-169 oC).

The ^{13}C NMR spectrum ($CDCl_3$) possessed twenty-six peaks and was consistent with the assigned structure.

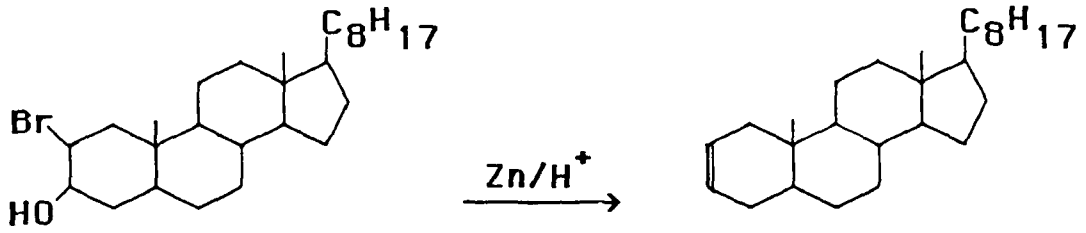
7. 2ß-Bromocholestan-3-ol



Sodium borohydride (0.59 g ,0.016 mol) was added to a suspension of 7.24 g (0.0155 mol) of 2ß-bromocholestan-3-one in 300 mL of ethanol which was maintained at 25 °C. All of the crystals dissolved after 2 h. After standing at room temperature for an additional 20 h, it was poured into 1.2 L of water. After remaining at 5 °C for 6 h, the white precipitate was removed by filtration, washed thoroughly with water, and dried *in vacuo* to yield 7.18 g of white solid, mp 100-102 °C. This solid was recrystallized from ethanol to afford 5.54 g (12.3 mmol, 80 % yield) of white solid, mp 101-103 °C (lit1 mp 104-105 °C).

The IR spectrum (KBr) exhibited a broad absorption at 3470 cm^{-1} (OH) and no carbonyl absorption. The ^{13}C NMR ($CDCl_3$) showed the following twenty-seven peaks: 76.20; 60.72; 56.30; 56.23; 54.06; 48.56; 44.68; 42.57; 39.83; 39.53; 39.44; 36.16; 36.08; 35.78; 34.94; 31.76; 28.22; 28.02; 27.95; 24.20; 23.83; 22.83; 22.57; 21.34; 18.68; 12.69; and 12.09 ppm.

8. Cholest-2-ene[19]



A mixture of 5.25 g (0.0112 mol) of 2ß-bromocholestan-3-ol and 10.5 g (0.161 g atm) of zinc dust in 150 mL of acetic acid was refluxed for 0.5 h, and the solution was filtered while hot. On cooling, the resulting solid was removed by filtration to give 3.80 g (10.3 mmol, 91.1% yield) of needle-like crystals, mp 71-73 °C (Ilt[21] mp 68-69 °C).

The ^{13}C NMR spectrum ($CDCl_3$) showed the following twenty-seven peaks: 125.96; 125.86; 56.53; 56.32; 54.11; 42.526; 41.49; 40.06; 39.82; 39.54; 36.21; 35.82; 35.66; 34.62; 31.867; 30.35; 28.81; 28.25; 28.03; 24.23; 23.86; 22.83; 22.58; 20.95; 18.71; 12.01; and 11.71 ppm.

B. 2,3-EPITHIOCHOLESTANE

The sequence presented above was repeated several times in order to provide a reasonable supply of cholest-2-ene. The next series of reactions involved the conversion of the alkene to the corresponding epoxide and, ultimately, to the episulfide.

1. Peroxybenzoic Acid[22]

Magnesium sulfate heptahydrate (0.5 g, 0.002 mol), sodium hydroxide (6.0 g, 0.15 mol) and 30% H_2O_2 (15 mL, 0.15 mol) were dissolved in 60 mL of water in a Teflon beaker[23] cooled to 20 °C . Methanol (75 mL) was added and the solution maintained at 20 °C. Powdered benzoyl peroxide (12.1 g, 50.0 mmol) was added in one portion to the mixture with stirring (Teflon-coated stirring bar). After 10 min the reaction mixture was poured into a 20% sulfuric acid solution (150 mL) and the resulting mixture extracted with three 50 mL portions of

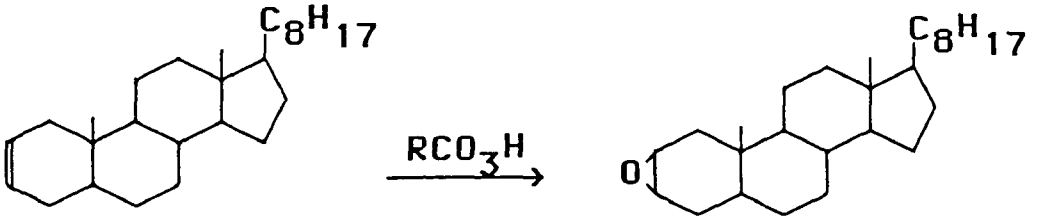
chloroform. The titration of 5 mL of the chloroform-peroxybenzoic acid solution required 46.6 mL of 0.1 N $Na_2S_2O_3$ solution.

1 mL of 0.1 N $Na_2S_2O_3$ = 0.0069g of peroxy acid

46.6 mL x 0.0069g/mL = 0.3215g of peroxy acid

0.3215 g/5 mL = 0.064g of peroxy acid/mL

2. 2,3-Epoxycholestane



Cholest-2-ene (12.24 g, 0.03302 mol) was dissolved in 100 mL of the perbenzoic acid-chloroform solution described above (0.064 g/mL, 0.0463 mol) at -10 °C for 36 h. The reaction mixture was mixed with an excess of ether and washed with 100 mL of cold, saturated aqueous sodium carbonate. The ether layer then was washed with water to pH 7, dried (sodium sulfate) for 30 min, filtered, and ultimately evaporated to dryness under reduced pressure. There resulted 15.93 g of crude, solid epoxide.

This solid was recrystallized from a mixture of ether-ethanol (8:2 v/v) to afford 13.24 g of a white, crystalline solid, mp 102-104 °C. This solid again was recrystallized from an ether-ethanol mixture. The first crop of crystals corresponded to 5.58 g (mp 106-107.5 °C) of solid. Concentration of the mother liquor afforded an additional 1.08 g of white solid, mp 97.5-100 °C (lit[24] mp 105 °C).

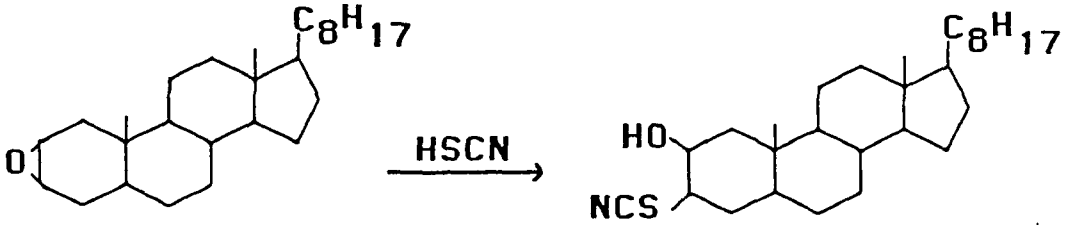
The ^1H NMR spectrum ($CDCl_3$) exhibited signals at 3.25-2.48 ppm (2H, m), and 2.19-0.19 ppm (48H, m). The ^{13}C NMR spectrum ($CDCl_3$) possessed the following signals: 56.27; 56.18; 55.91; 53.67; 52.57; 52.39; 52.20; 51.03; 46.72; 42.60; 42.352; 39.87; 39.48; 38.25; 36.19; 36.13; 35.75; 35.65; 35.35; 33.98; 33.57; 31.88; 31.63; 30.37; 29.03; 28.43; 28.16; 27.98; 26.82; 24.18; 24.10; 23.79; 22.79; 22.55; 21.29; 20.85 18.64; 13.45; 12.90; 12.11; 11.91; and 14.20 ppm.

3. Thiocyanic Acid

$$KSCN + H^+ \text{ -----> } HSCN + K^+$$

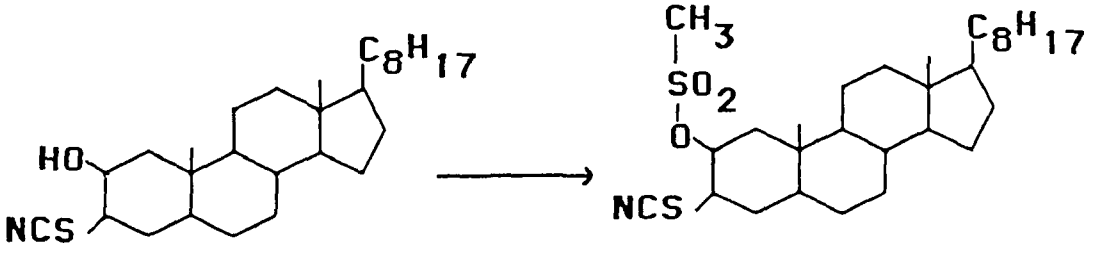
In a 250 mL separatory funnel there was placed 20.0 g of potassium thiocyanate (0.328 mol) dissolved in a small amount of ice-water and 100 mL of ethyl ether. The mixture was shaken and 18 mL of conc. phosphoric acid added in small portions. The ether layer was washed with a small amount of water, dried (sodium sulfate), the drying agent removed by filtration and the ethereal solution used for the following reaction.

4. 3-Thiocyanatocholestan-2-ol



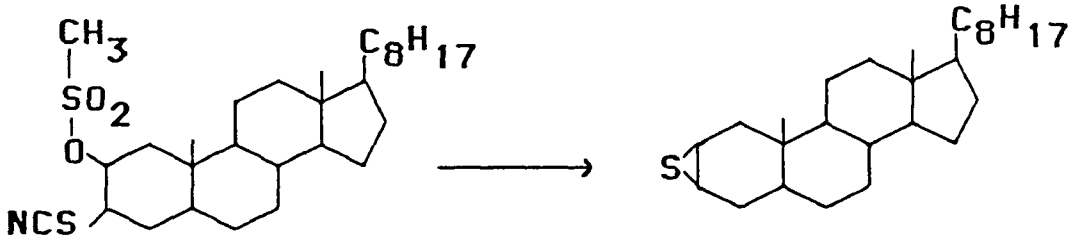
To the above HSCN-ether solution there was added 2.52 g (0.00653 mol) of 2,3-epoxycholestane. This solution was allowed to stand at room temperature for 66 h. The ether solution then was washed with an aqueous sodium carbonate solution, water, and then dried (sodium sulfate). The solvent was removed under reduced pressure. The solid residue was recrystallized from methanol to afford 1.92 g of a white solid, mp 143-147 °C. The IR spectrum (KBr) showed an OH absorption at 3490 cm-1, and a CN absorption at 2160 cm-1.

5. 3-Thiocyanato-2-cholestanyl Mesylate



To a solution of 0.68 g (1.5 x 10-3 mol)) of 3-thiocyanatocholestan-2-ol in 20 mL of dried pyridine there was added 1 mL of mesyl chloride with cooling. The mixture was stored overnight in a refrigerator. After coming to room temperature, the product was extracted with ethyl ether. The ether extract was washed with water to neutrality, dried (sodium sulfate) and then evaporated to dryness. The residue (0.81 g) was recrystallized from petroleum ether to yield 0.15 g of crystals. The TLC[25] of these crystals showed only one spot. The ^1H NMR spectrum ($CDCl_3$) included a singlet (CH_3) at 3.0 ppm.

6. 2,3-Epithiocholestane



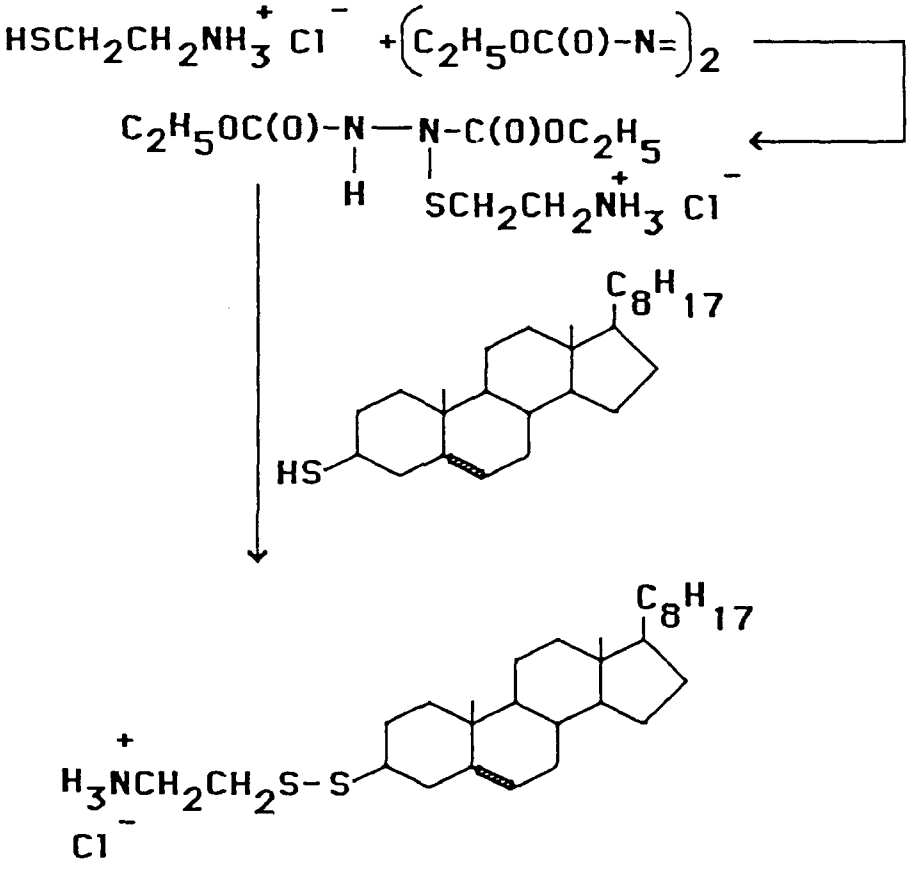
A solution of 7.21 g (0.014 mol) of 3-thiocyanato-2-cholestanyl mesylate in 100 mL of ethyl ether was added to 500 mL of boiling 3 % KOH-MeOH solution and heated under reflux for 0.5 h. Water (400 mL) was added and the resulting precipitate removed by filtration. The resulting solid was dried *in vacuo* to yield 4.8 g of solid, mp 101-105 °C. The solid was recrystallized from chloroform-methanol to yield 3.88 g of solid, mp 107-109 °C.

This solid (1.00 g) was column chromatographed over alumina (Activity I, neutral, 70 g) with petroleum ether-benzene eluent (9:1 v/v) to afford 0.68 g of a solid. Recrystallization from acetone afforded 0.52 g of white solid, mp 116-119 °C. The ^{13}C NMR spectrum ($CDCl_3$) showed signals at 56.23; 56.24; 43.09; 42.46; 39.98; 39.50 39.41; 37.08; 36.17; 35.70; 35.45; 34.76; 34.52; 31.84; 30.19; 28.49; 28.18; 27.99; 24.18; 23.83; 22.81; 22.55; 20.75; 18.67; 14.82 and 12.00 ppm.

While this material appeared to be rather pure episulfide,[26] it was not used for any further reactions due to a change in focus away from steroid-based radioprotectives.

C. CHOLESTERYL CYSTEAMINYL DISULFIDE HYDROCHLORIDE

During the first year of this contract this compound was submitted for bioassay. It's synthesis was accomplished by the "thiolsulfinate + thiol" route. We were, however, interested in the possibility of preparing such steroid-based mixed disulfides using the DEAD reaction. Our goal was to assess which of these routes was the more valuable in preparing such mixed disulfides.



In a 100 mL three-necked flask equipped with a condenser, an addition funnel and a nitrogen inlet tube there was placed 1.74 g (0.0100 mol) of diethyl azodicarboxylate dissolved in 10 mL of ethanol. The reaction flask was kept in an ice-salt bath to maintain the temperature below 0 °C all the time. Cysteamine hydrochloride 1.13 g (0.0100 mol) was dissolved in 25 mL of ethanol and this mixture was added dropwise to the reaction flask over a period of 0.5 h. The color of the solution changed from orange to yellow.

Thiocholesterol 4.02 g (0.0100 mol) was dissolved in ethanol-chloroform mixed solvent and this solution added dropwise to the reaction mixture over a period of 0.5 h. The color of the solution changed from yellow to colorless; a white precipitate formed. Stirring was continued for an additional 3 h. All

volatiles were removed under reduced pressure to afford 7.70 g of a white solid .

This solid (7.20 g) was suspended in 400 mL of acetone and 5.20 g of undissolved solid was separated by filtration. This solid was recrystallized from ethanol to afford 3.08 g of white precipitate, mp 190-191°C. A second crop (0.36 g , mp 188-190 oC) was collected after the volume of the filtrate was further reduced. The final yield was 71.4%.

The HPLC[27] of the sample melting 190-191 °C showed the presence of a single peak with retention time 2.5 min. Mp and NMR were consistent with an authentic sample which had previously been prepared in this laboratory.

IV-4. NON-STEROID, NON-POLYMER COMPOUNDS

This program resulted in the synthesis and submission of five compounds which (a) did not contain a steroid nucleus and (b) did not contain a polymer of some type. The preparation of these five compounds are described below.

A. *CYSTEAMINYL 4-HYDROXYPHENYL DISULFIDE HYDROCHLORIDE*

$$\text{-Cl} + NH_3(CH_2)_2SH + [EtOC(O)N]_2 \longrightarrow EtO_2CNHN(SCH_2CH_2NH_3^+\ Cl^-)CO_2Et$$

$$EtO_2CNHN(SCH_2CH_2NH_3^+\ Cl^-)CO_2Et + p\text{-}HOC_6H_4SH \longrightarrow [EtOC(O)NH]_2 + p\text{-}HOC_6H_4SSCH_2CH_2NH_3^+\ Cl^-$$

In a three-necked flask, equipped with a condenser, addition funnel and gas inlet adapter there was placed 26.13 g (0.15 mol) of DEAD dissolved in 60 mL of absolute ethanol. The flask was immersed in an ice-salt bath and cooled to 0 to -10 oC. To this there then was added, dropwise and with stirring, a solution of cysteamine hydrochloride (17.04 g, 0.15 mol) dissolved in 125 mL of absolute ethanol. Addition required 2 h. During this time the color of the solution changed to yellow. Stirring was continued for an additional 10 h.

To this there then was added 18.93 g (0.150 mol) of p-hydroxythiophenol dissolved in 50 mL of absolute ethanol. Addition required 2 h. During this time the solution became colorless and a white precipitate was formed. After addition had been completed stirring was continued for an additional 2 h. The resulting white precipitate was removed by filtration and the filtrate concentrated to dryness under reduced pressure. The solid resulting from this concentration (43.46 g) was extracted with ether overnight using a Soxhlet extractor. The ether-insoluble material remaining in the thimble was dried to afford 20.37 g of an off-white solid. This solid was recrystallized from ethanol to afford 10.58 g (0.0445 mol, 29.7 % yield) of white solid, mp 171-172 oC. HPLC[28] of the solid

showed a single peak with retention time 23 min. The proton resonance spectrum of this material was consistent with that of the desired product.

ANAL. Calcd for $C_8H_{12}NOS_2Cl$: C, 40.41 %; H, 5.09 %; N, 5.89 %; S, 26.97 %
Found: C, 40.25 %; H, 5.05 %; N, 5.92 %; S, 26.86 %

B. CYSTEAMINYL 10-CARBOXYLDECYL DISULFIDE HYDROCHLORIDE

The preparation requires the synthesis of the thiol of undecanoic acid. This is accomplished by converting a bromide into an isothiouronium salt. Neither the intermediate ester nor the intermediate isothiouronium salt are ever isolated. The alkaline hydrolysis of the salt occurs simultaneously with the alkaline hydrolysis of an ester group.

1. 11-Mercaptoundecanoic Acid

$$Br\text{-}(CH_2)_{10}\text{-}CO_2CH_3 + (NH_2)_2C{=}S \dashrightarrow \dashrightarrow HS\text{-}(CH_2)_{10}\text{-}CO_2H$$

A mixture of 24.90 g (0.0892 mol) of methyl 11-bromoundecanoate, 6.79 g (0.0892 mol) of thiourea and 50 mL of methanol was refluxed for 3 h. A solution of 8.00 g (0.20 mol) of NaOH in 50 mL of water was added, and the mixture refluxed for 3 h. During this period the mercaptan separated as an oil. The reaction mixture was acidified with dilute hydrochloric acid (10 mL of concentrated HCl to 50 mL of water) and then extracted with 75 mL of hot benzene solution. The extract was washed with 50 mL of water and dried over anhydrous magnesium sulfate. The drying agent then was removed by filtration and the solvent removed using a rotary evaporator. The residue was recrystallized from benzene to yield 10.01 g (54.0 %) of a white solid, mp 92-95 °C. This solid gave a positive result (yellow color) with Ellman's reagent. The ^1H and ^{13}C NMR spectra (CF_3COOH) were consistent with the assigned structure.

2. *Cysteaminyl 10-Carboxyldecyl Disulfide Hydrochloride*

$$HSCH_2CH_2NH_3^+ Cl^- + EtOC(O)N{=}NCOOEt \dashrightarrow EtOC(O)NH\text{-}N(SCH_2CH_2NH_3^+ Cl^-)COOEt$$

$$EtOC(O)NH\text{-}N(SCH_2CH_2NH_3^+ Cl^-)COOEt + HS\text{-}(CH_2)_{10}\text{-}COOH \dashrightarrow$$

$$EtO_2CNH\text{-}NHCO_2Et + HO_2C(CH_2)_{10}SSCH_2CH_2NH_3^+ Cl^-$$

In a 250 mL three-necked flask equipped with an addition funnel and a nitrogen inlet tube there was placed diethyl azodicarboxylate (DEAD) (8.19 g, 0.0469 mol) in 25 mL of ethanol. Cysteamine hydrochloride (5.34 g, 0.0469 mol) was dissolved in 100 mL of chloroform and the solution added, dropwise and with stirring, to the reaction flask; addition required 1 h. During this time the temperature was kept at -5 °C to 0 °C and the color changed from orange to yellow.

11-Mercaptoundecanoic acid (10.24 g, 0.0469 mol) was dissolved in 100 mL of ethanol and added, dropwise and with stirring, to the reaction flask. The temperature was maintained at -5 °C during the addition of the thiol. The color changed from yellow to colorless and a white precipitate formed. Stirring was continued for 8 h. The resulting precipitate 10.15 g was removed and the filtrate concentrated to dryness under reduced pressure to yield 13.24 g of a white solid.

A sample (1.03 g) of the initially-formed solid was recrystallized from a chloroform-methanol mixture to yield 0.63 g of a white solid. The ^{1}H NMR spectrum (pyridine-d_5) exhibited signals at 3.9 ppm (2H, t), 3.7 ppm (2H, t), 2.9 ppm (2H, t), 2.7 ppm (2H, t),and 2.0-1.0 ppm (16H, m). The ^{13}C NMR showed nine peaks: 39.23 ppm, 38.89 ppm, 35.38 ppm, 34.89 ppm, 29.59 ppm, 29.33 ppm, 28.64 ppm, 25.58 ppm, and 177.75 ppm. The TLC (8" silica gel plate with methanol and ethanol as eluent) showed only one spot. The IR spectrum (KBr) demonstrated the presence of $-NH_3+$ (3300 cm^{-1}) and C=O (1750 cm^{-1}) groups.

An additional 4.08 g from the 10.15 g sample was recrystallized from methanol to yield 2.90 g of white solid, and this then recrystallized from methanol to afford 2.36 g (38.0 %) of a white solid, mp 132-134 °C. The ^{1}H NMR spectrum (CF_3CO_2D) showed signals at 4.508 ppm (2H, t), 3.841 ppm (2H, t), 3.509 ppm (2H, t), 3.341 ppm (2H, t), 2.546 ppm (2H, t), and 2.172 ppm (14H, m). The ^{13}C NMR showed twelve peaks: 185.241; 41.251; 39.990; 35.694; 35.122; 30.957; 30.876; 30.691; 30.610; 30.037; 29.991; and 26.453 ppm. The HPLC showed a single peak with retention time 3 min. The IR spectrum (KBr) demonstrated the presence of $-NH_3+$ (3300 cm^{-1}) and C=O (1750 cm^{-1}) groups.

ANAL. Calcd for $C_{13}H_{28}ClNO_2S_2$: C, 47.32 %; H, 8.55 %; N, 4.25 %; S,19.43 %
Found: C, 47.47 %; H, 8.67 %; N, 4.24 %; S,19.43 %

C. 2-MERCAPTOPHENOTHIAZINE

1. Copper[29]

Cupric sulfate pentahydrate (10.0 g, 4.005 x 10-2 mol) was dissolved in 35 mL of hot water. After the solution had cooled to room temperature, zinc dust (3.50 g, 5.35 x 10-2 g atm) was gradually added, with stirring. Dark brown copper powder precipitated . This solid was washed with three 100 mL portions of water and then with 30 mL of dilute (5%) hydrochloric acid. [30] This acidic

solution was decanted, and the copper powder washed with three 50 mL portions of water. The resulting copper was stored under water. [31]

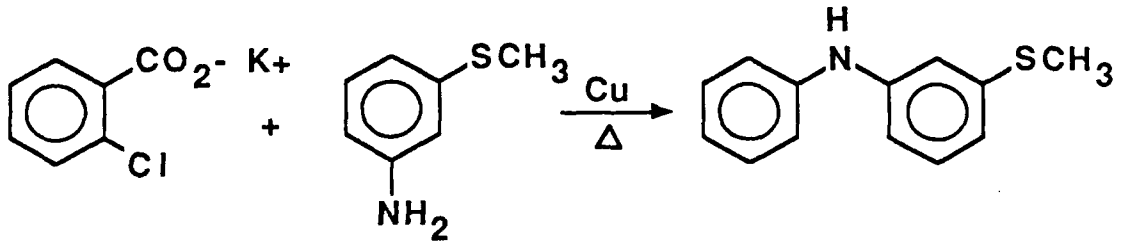
2. Potassium 2-Chlorobenzoate

Potassium hydroxide (42.21 g, 85% pure, 0.641 mol) was dissolved in 100 mL of water. To this there was added 100.0 g (0.639 mol) of o-chlorobenzoic acid suspended in 20 mL of water.

After heating on a steam bath for 0.5 h, the suspension was filtered hot to remove some undissolved brown solid. The clear, light yellow filtrate was placed in a 500 mL r.b. flask containing 220 mL of benzene and equipped with a Dean-Stark trap. This mixture was refluxed until no more water was deposited in the Dean-Stark trap. The resulting solid (paste) was dried *in vacuo* at 50 °C, to afford 99.5 g of an off-white powder, mp 235-237 °C. The solid was purified by dissolving it in the minimum amount of hot water and then adding acetone to the cooled, aqueous solution. The resulting solid was separated by filtration and dried at 3.5 torr and 50 °C for 12 h. There resulted 90.7 g (0.466 mol, 73% yield) of the desired salt, mp 239-241.5 °C.

The proton nmr of this material (D_2O, DSS standard) exhibited a complex multiplet at 7.15-7.50 ppm. The sample was homogeneous on TLC (8" silica gel; chloroform eluent; uv and iodine visualization).

3. 2-Carboxyl-3'-Methylmercaptodiphenylamine

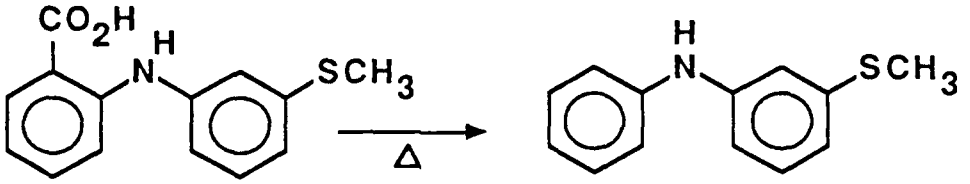


Wet copper powder (synthesis described above) was placed in a weighing bottle and rinsed successively with acetone and with hexane. After removing the solvent by decantation, the solid was dried *in vacuo* .

In a 1000 mL three-necked flask, equipped with an overhead stirrer, condenser and nitrogen gas inlet were placed potassium 2-chlorobenzoate (51.13 g, 0.2627 mol), 3-methylmercaptoaniline (40.24 g, 0.2891 mol), 2.20 g (0.0346 g atm) of dried copper powder and 170 mL of 4-methyl-2-pentanol. The reaction mixture was refluxed for 1.5 h. TLC[32] of the reaction mixture indicated that the reaction was nearly complete. After allowing the mixture to cool, 310 mL of 2N sodium hydroxide solution was added and the mixture was steam distilled until a second layer was not produced.[33]

Activated carbon (≈ 5 g) was added to the residue and the carbon then removed by filtration. To the filtered solution there was added 20 g of sodium chloride and 120 mL of 18% hydrochloric acid, slowly and with stirring. The resulting gray precipitate was removed by filtration. This solid then was dissolved in acetone and the resulting solution dried with magnesium sulfate. After removal of the drying agent the solvent was removed under reduced pressure to afford 49.6 g of the desired product as a gray solid, mp 119-132 °C. Two recrystallizations from ethanol yielded 30.15 g (60.74% yield) of the title compound as brown crystals, mp 139-141 °C (lit[34] mp 139-141°C). An additional 4.34 g (0.0167 mol, 6.37%) of product was obtained by concentrating the mother liquors.

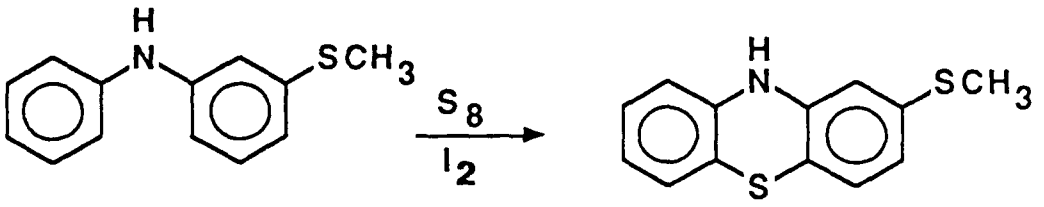
4. 3-Methylmercaptodiphenylamine



2-Carboxy-3'-methylmercaptodiphenylamine (31.08 g, 0.1198 mol) was placed in a 100 mL flask equipped with an overhead stirrer and a nitrogen inlet. The flask was heated in a Woods metal bath at 250-260 °C for 45 min to afford a dark, oily residue. A TLC (silica gel substrate, benzene eluent, iodine visualization) of the residue indicated only a small amount of unreacted starting material. Upon cooling to room temperature, this oil solidified to a solid, mp 39-43 °C. The resulting solid was dissolved in 105 mL of chloroform and the resulting solution washed with 52 mL of 1N ammonium hydroxide solution and then with 18 mL of water. The chloroform solution was dried (magnesium sulfate) and the drying agent removed by filtration. Evaporation of the solvent under reduced pressure afforded 22.99 g (0.1068 mol, 89.12% yield) of the crude product. This was distilled to afford 20.79 g (0.09655 mol, 80.59% yield) of the desired product, bp 143-149 °C (0.08-0.09 torr). This oil solidified at room temperature to afford a tan solid, mp 57-59 °C (lit[33] mp 59-61 °C). This sample was homogeneous to TLC (8" silica gel, benzene eluent, iodine visualization).

The 1H NMR spectrum of this substance displayed absorptions at 2.45 ppm (singlet, CH3), 5.77 ppm (broad singlet, NH), and 6.73-7.32 ppm (multiplet, Ar-H).

5. 2-Methylmercaptophenothiazine



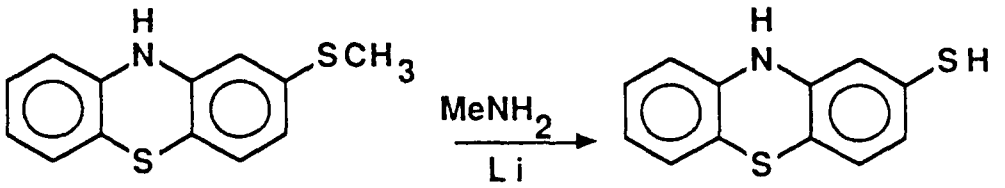
In a 250-mL three-necked flask equipped with an overhead stirrer, nitrogen gas inlet and condenser, 90.63 g (0.4209 mol) of 3-methylmercaptodiphenylamine was mixed with 26.97 g (0.8412 g atm) of sulfur and 1.20 g (4.73 x 10-3 mol) of iodine. The mixture was placed in an oil bath, the temperature raised to 160 °C during one hour and then maintained at 160 °C for another hour; by this time the evolution of gas (hydrogen sulfide) from the flask had ceased. TLC (8" silica gel , benzene eluent, uv and iodine visualization) indicated that all of the diphenylamine had reacted. The reaction mixture was recrystallized from 100 mL of xylene. The resulting crystals were collected by filtration and dried overnight in a vacuum desiccator to afford 74.95 g (73.54% crude yield) of green powder, mp 119-125 °C (lit[33] mp 138-140 °C).

The green powder was dissolved in 600 mL of hot ethanol and this was followed by the addition of 6.00 g of activated charcoal (Nuchar). The charcoal was removed by hot filtration. The crystals which formed upon cooling were collected by filtration. These then were dried overnight in a vacuum desiccator attached to mechanical pump (potassium hydroxide drying agent) to afford 49.89 g of light green powder, mp 130-134 °C. This powder was recrystallized from 500 mL of ethanol (decolorized with 1.5 g of Nuchar) to afford 40.75 g (0.1661 mol, 39.46% yield) of 2-methylmercaptophenothiazine, mp 136-139 °C.

The product was homogeneous to HPLC.[35] The IR spectrum (Nujol) of the product was consistent with the structure. The NMR spectrum of product could not be acquired in chloroform solution due to the decomposition. The ^1H NMR spectrum in acetone-d_6 displayed absorptions at 2.34 ppm (singlet), 6.61-7.02 ppm (complex multiplet) and 7.77 ppm (broad singlet). The ^{13}C NMR spectrum (acetone-d_6) displayed absorptions at 15.53, 113.05, 114.86, 115.42, 118,38, 120.73, 122.91, 127.09, 127.33, 128.16, 138.71, 142.83, and 143.64 ppm.

This reaction was conducted a total of four times. The maximum yield of purified product was 43%.

6. *2-Mercaptophenothiazine*[36]



To a 500-mL three-necked flask equipped with overhead stirrer, gas inlet and Dewar condenser filled with Dry Ice-isopropanol mixture there was added 15.00 g (0.06113 mol) of 2-methylmercaptophenothiazine. Methylamine gas was passed through a drying tube filled with potassium hydroxide pellets and condensed in the flask until 400 mL of liquid methylamine was collected. The system then was covered with a nitrogen blanket. Lithium wire (0.87 g, 0.13 g atm) was placed in a Soxhlet extractor which then was inserted between the Dewar condenser and the flask. The lithium was slowly added into the flask over a period of 3 h by refluxing the methylamine. The color of the reaction mixture changed from green to brown to dark red. HPLC[37] indicated the presence of some unreacted sulfide.

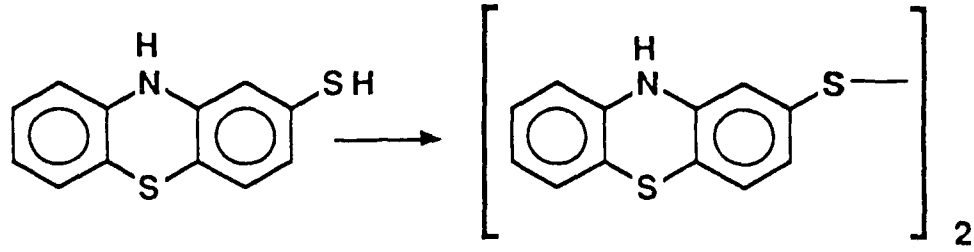
Another 0.56 g (0.081 g atm) of lithium wire was added. HPLC[36] indicated the absence of sulfide. The reaction was quenched by adding 40 mL of methanol. The methylamine was evaporated under a nitrogen atmosphere and this was followed by the addition of 200 mL of water. The basic solution was extracted with 200 mL of ether and then acidified with 100 mL of 18% of hydrochloric acid (pH = 2 Hydrion paper). A light yellow solid precipitated out of solution and was extracted into ether (400 mL, 2 x 200 mL, 2 x100 mL). The ether solution was evaporated under reduced pressure and the residue dried overnight in a vacuum desiccator (potassium hydroxide drying agent, mechanical pump) to afford 11.77 g (83.23% crude yield) of a yellow powder.

The ^1H NMR spectrum ($CDCl_3$) of the crude product displayed no -SCH_3 signal. The crude product was recrystallized twice from benzene (dry box) to afford 3.16 g (0.0137 mol, 22.3% yield) of 2-mercaptophenothiazine, mp 212-214 °C (lit mp 211-213 °C) which was homogeneous to HPLC (same conditions as above). The IR spectrum (Nujol) displayed an absorption at 2563 cm-1 (S-H stretch). The ^1H NMR spectrum (acetone-d_6) displayed absorptions at 4.20 ppm (singlet), 6.68-7.04 ppm (complex multiplet) and 7.83 ppm (broad singlet). The ^{13}C NMR spectrum exhibited signals at 115.44, 118.25, 122.95, 123.34, 127.04, 127.50, 128.19, 131.25 142.72 and 143.77 ppm. The band-width at half-height indicated that the absorptions at 115.44 and 128.19 ppm probably contained two carbons.

ANAL. Calcd for $C_{12}H_9NS_2$: C, 62.30%, H, 3.92%; N, 6.60%; S, 27.72%
Found: C, 26.21%; H, 3.70%; N, 5.90%; S, 27.54%

This sequence has been conducted a total of six times.

D. *2-PHENOTHIAZINYL DISULFIDE*



Using the same procedure described above, 10.00 g (0.4075 mol) of 2-methylmercaptophenothiazine was reduced by lithium in methylamine to yield 6.90 g of crude 2-mercaptophenothiazine.

In a 500-mL three-necked flask equipped with an overhead stirrer, condenser and addition funnel, 5.56 g of this crude product was mixed with 300 mL of ethanol and 5 mL of triethylamine. The mixture then was heated to reflux. Iodine (1% solution in ethanol) was added into the flask until the iodine color persisted in the flask. A brown solid which precipitated was collected by filtration and dried (vacuum desiccator, potassium hydroxide) for 3 days. This afforded 5.24 g (92.0% crude yield) of a yellow solid which did not give a positive test for thiol. This yellow solid was suspended in 800 mL of refluxing chlorobenzene. The undissolved solid was removed by filtration and suspended in 2.5 L of refluxing xylene; the undissolved solid was removed by hot filtration. Upon cooling, yellow, plate-like crystals formed in both the chlorobenzene and the xylene solutions. The crystals were collected, combined and then dried in an Abderhalden drying pistol (phosphorus pentoxide drying agent) overnight to afford 2.43 g (0.00528 g, 22.0% yield) of 2-phenothiazinyl disulfide, mp 298-300.5 °C (lit[33] mp 277-279 °C).

This sample was homogeneous to TLC[38]. The ^{1}H NMR spectrum (dimethylformamide-d_7) displayed absorptions at 6.60-7.40 ppm (complex multiplet) and 8.75 ppm (broad singlet). The ^{13}C NMR spectrum (dimethylformamide-d_7) displayed absorptions at 113.51, 115.29, 117.28, 117.63, 121.45, 122.82, 126.88, 127.36, 128.26, 136.23, 142.44 and 144.03 ppm.

ANAL. Calcd for $C_{24}H_{16}N_2S_4$: C, 62.57%, H, 3.50%, N, 6.08%; S, 27.84%
Found: C, 62.76%; H, 3.15%; N, 5.80%; S, 28.06%

E. CYSTAMINE S-OXIDE DIHYDROCHLORIDE

The S-oxides of disulfides (RS(O)SR) are sometimes termed thiolsulfinates. The preparation of this thiolsulfinate, the first which we have submitted under this contract, begins with the preparation of the oxidant peroxybenzoic acid. This, in turn, is used to oxidize cystamine dihydrochloride.

1. Peroxybenzoic Acid

A sample of 0.518 g (0.00200 mol) of magnesium sulfate heptahydrate, 6.0 g (0.15 mol) of sodium hydroxide and 15 mL (0.15 mol) of 30% aqueous hydrogen peroxide was dissolved, sequentially, in 90 mL of water in a 500 mL Teflon beaker. Care was taken to ensure that the temperature of the solution did not rise above 20 °C by placing the beaker in an ice bath. To this there was added (slowly!) 115 mL of methanol, the temperature being kept several degrees below 25 °C during the addition. Powdered benzoyl peroxide (12 g, 0.075 mol) then was added in one portion and the resulting suspension stirred for 15 min at 20 °C. The reaction mixture then was poured into 250 mL of ice cold 20% H_2SO_4 and mixed well.

This cold solution was extracted with three 50 mL portions of chloroform. The chloroform fractions were collected and dried over anhydrous magnesium sulfate. The solution was filtered, stored in a plastic bottle and refrigerated for later use.

The concentration of peroxybenzoic acid was determined as follows. A 50 mL aqueous solution was prepared containing 1.5 g (0.010 mol) of sodium iodide, 5 mL of glacial acetic acid and 5 mL of chloroform. To this solution there was added 1.0 ml peroxybenzoic acid described above. The resulting mixture became dark (molecular iodine). This solution then was titrated to the colorless endpoint using a 0.1 M sodium thiosulphate solution (9.95 mL). Two additional titrations yielded 9.90 mL and 9.93 mL endpoints. From the relationship: 0.0069 g perbenzoic acid = 1 mL (0.1 M) sodium thiosulphate, the concentration of peroxybenzoic acid was calculated to be 0.0684 g/mL (9.93 ml • 0.0069/1 mL)

2. *Cystamine S-Oxide Dihydrochloride*

A 500 mL, 3-necked flask was equipped with a mechanical stirrer and an addition funnel. A sample of 11.5 g (0.0503 mol) of cystamine dihydrochloride and 250 mL of anhydrous methanol was placed in the flask. A solution of 95 mL of peroxybenzoic acid (described above), containing 6.5 g (0.047mol) of peroxybenzoic acid, was placed in the addition funnel. This solution was added, dropwise, at room temperature over a period of 1 hr. The reaction mixture was stirred overnight resulting in the formation of a white precipitate. This solid was removed by filtration and dried in a vacuum dessicator (0.01 mm Hg) overnight to yield 2.3 g of crude product, mp 147-150 °C (dec). The IR (KBr) showed a pronounced S-O stretch at 1055 cm^{-1}.

The filtrate was reduced to half of its original volume using a rotary evaporator. To the resulting solution there was added 5 mL of the peroxybenzoic acid solution described above (containing 0.342 g perbenzoic acid). This mixture was stirred overnight to yield 3.1 g of a white solid, mp 147-149 °C (dec). The 1H and 13C NMR spectra (D_2O) of these solids showed the

presence of a small amount of unreacted starting material (^1H triplet at 2.98 ppm and ^{13}C resonances at 40.40 and 35.97).

Washing the 2.3 g sample with 25 mL of hot ethanol gave 1.97 g of a white solid, mp 147-149 °C (dec), whose proton NMR (D_2O) did not show the presence of any starting material.[39] The remaining solid was treated similarly and the resulting solid combined with the material melting 147-149 °C (dec). This combined sample was washed with 25 mL of hot ethanol to yield 2.67 g of a white solid, mp 147-149 °C (dec). The ^1H NMR spectrum of of this solid showed a complex multiplet around 3.5 ppm. The ^{13}C NMR spectrum showed the presence of four carbons (53.56, 42.29, 37.02 and 32.83 ppm. The HPLC[40] analysis showed the sample to be homogeneous.

The volume of the washing was reduced in half using a rotary evaporator. To this there was added 5 mL of peroxybenoic acid (described above, containing 0.342 g perbenzoic acid) and the resulting solution was stirred overnight. This resulted in the formation of 3.82 g of white solid , mp. 159-161 °C (dec).

ANAL.

Calcd for $C_4H_{14}N_2OS_2Cl_2$: C,19.91%; H, 5.84%; N, 11.61%; S, 26.59%; Cl, 29.40%.
Found: C, 20.18%; H, 5.72%; N, 11.36%; S, 25.30%; Cl, 29.46%

IV-5 POLYMER-BOUND RADIOPROTECTIVE AGENTS

As part of this program, it was decided to evaluate the feasibility of binding radioprotective agents to polymers of various types. Such polymer-bound systems might serve as points of departure for developing novel methods of delivering radioprotective agents either by implantation or else via transdermal methods. These polymers also were synthesized in an attempt to determine if binding an aminothiol to a polymer via its amino group would provide some measure of stability towards the oxidation of the mercapto group.

Radioprotective agents may be bound either by ionic bonds or by covalent bonds to polymers. In the following three sequences, a small aminothiol, or similar compound, is bound by an ionic bond to a polymer. In each instance the ionic bond is between an anionic center on the polymer and an ammonium cation in the radioprotective agent. The polymer selected was a commercially-available ion-exchange resin. This polymer, as shown below, lacks any nitrogen. Consequently, the binding of aminothiols to this polymer could be evaluated by elemental analyses.

A. ACTIVATION OF DOWEX-50W

A 10.0 g sample of Dowex 50W-X8 was washed, sequentially, with 500 mL of 1 N sodium hydroxide, 500 mL of 1 N hydrochloric acid, 500 mL of 1 N sodium hydroxide, 500 mL of 1 N hydrochloric acid, 500 mL of 1 N sodium

hydroxide and, finally, with distilled water until the filtrate was neutral (Hydrion paper). The polymer sample then was air dried to yield 14.7 g of resin beads. Elemental analysis for nitrogen showed 0.00 % present in this resin.

B. *DOWEX-BOUND CYSTEAMINE*

A 5.07 g sample of resin was placed in a 50 mL flask and to this there was added a 25 mL of water containing 2.5 g (0.022 mol) of cysteamine hydrochloride. The suspension was mixed and allowed to stand for 2 h. The treated resin beads were removed by filtration and washed with 50 mL of distilled water and then air dried to give 6.91 g of beads. This sample was dried in an Abderhalden drying apparatus (phosphorous pentoxide drying agent; acetone reflux; 0.02 mm Hg) for 4 h. This yielded 3.25 g of a resin whose elemental analysis showed the presence of 4.11 % of nitrogen.

C. *DOWEX-BOUND WR-1065*

A 5.0 g sample of resin (described in **A**, above) was placed in a 50 mL flask and to this there was added 25 mL of an aqueous solution containing 1.5 g (0.0072 mol) of 2-((3-aminopropyl)amino)ethanethiol dihydrochloride. The suspension was mixed and allowed to stand for 2 h. The resin then was removed by filtration and washed with 50 mL of distilled water and air dried to give 4.7 g of beads. This sample then was dried in an Abderhalden drying apparatus (phosphorous pentoxide drying agent; acetone reflux; 0.02 mm Hg) for 4 h. There resulted 3.06 g of resin whose elemental analysis showed the presence of 3.60 % of nitrogen.

D. *DOWEX-BOUND CYSTAMINE*

A 4.63 g sample of resin (described in **A**, above) was placed in a 50 mL flask and to this there was added 25 mL of an aqueous solution containing 1.5 g (0.0067 mol) of cystamine dihydrochloride. The suspension was mixed and allowed to stand for 2 h. The resin was removed by filtration, washed with 50 mL of distilled water and then air dried to give 4.2 g of a resin. This sample was dried in an Abderhalden drying apparatus (phosphorous pentoxide drying agent; acetone reflux; 0.02 mm Hg) for 4 h. There resulted 3.25 g of resin whose elemental analysis showed the presence of 4.27 % of nitrogen.

V. FOOTNOTES AND REFERENCES

[1]The phenothiazines were prepared as an entry into the problem of protection of the CNS.

2 D. A. Swann and J. H. Turnbull, *Tetrahedron*, **20**, 1265 (1964).

3 Silica gel; 8"; chloroform solvent; I_2 and Ellman's reagent visualization.

4 P. A. Bobbio and F. O. Bobbio; *Chem. Ber.*, **95**, 2747 (1962).

5 Column: SI-5; hexane solvent; 1 mL/min flow rate; UV detector 220 nm; detector output 0.05 Au/mV; recorder input 10 mV; chart speed 2 cm/min; pressure 46 atm.

6 K. Freudenberg and W. Hess, *Ann.*, **448**, 128 (1926).

7 L. C. King, R. M. Dodson and L. A. Subluskey, *J. Amer. Chem. Soc.*, **70**, 1176 (1948).

8 Silica gel; 8"; chloroform eluent; iodine visualization.

9Column: SI-5, flow 1 mL/min; UV detector 220 nm; detector 0.1 Au/mV; chart 2 cm/min; input 10 mV; pressure 53 atm.

10 Aldrich Chemical Company.

11R. P. Volane, *Tetrahedron Letters*, **22**, 3119 (1981).

12Longer reflux periods, *e.g.*, 3 h, led to substantially lower yield of final product.

13It required this much time for the presence of unreacted steriod to no longer be detected using TLC (8" strips silica gel in ethanol using I_2 visualization).

14Column CN-5 using 85/15 chloroform/methanol as eluent at 1.0 ml/min. UV detection at 260 mn

15 8" silica gel plate; methylene chloride eluent; iodine visualization.

16 (a) *Helv. Chim. Acta*, **59**, 2821 (1976); (b) *J. Amer. Chem. Soc.*, **73**, 5252 (1951).

17 *Helv. Chim. Acta*, **59**, 2821 (1976).

18 8" silica gel plate; chloroform and hexane eluents; iodine visualization.

19 *Helv. Chim. Acta*, **59**, 2821 (1976).

20 L. F. Fieser and X. A. Dominguez, *J. Amer. Chem. Soc.*, **75**, 1705 (1953).

21 *J. Amer. Chem. Soc.*, **73**, 5252 (1951).

22 Y. Ogata and Y. Sawaki, *Tetrahedron*, **23**, 3327 (1967).

23 Use of a glass container appears to reduce the final yield of peroxyacid.

24 *Helv. Chim. Acta*, **32**, 275 (1949).

25 8" Silica gel plate; benzene or chloroform eluent; uv visualization.

[26] The melting points of the stereoisomeric episulfides are reported as 123-125 °C [3*á*] and 113-115 °C [3*ſ*] (A. Lightner and C. Djerassi, *Chem. and Ind.*, 1236 (1962).

[27]Pressure 66 atm, flow rate 1.0 mL/min, uv detecter 260 nm, chloroform:methanol (85:15 v/v).
[28]Methanol:water (75:25), pressure 194 atm, uv 254 nm, flow rate 0.5 mL/min.
[29] The use of several different commercially-available copper powders did not give acceptable yields. This procedure is based upon that in Organic Synthesis, Coll. Vol. II, A. H. Blatt, Ed., J. Wiley and Sons, New York, 1943, p.446.
[30]Employed to remove excess zinc.
[31]Exposure to air led to a rapid loss in activity.
[32] 8" silica gel plate; ethanol eluent; iodine visualization.
[33]Steam distillation was employed to remove unreacted 3-methylmercaptoaniline as well as 4-methyl-2-pentanol.
[34]J.P. Bourquin, G. Schwarb, G. Gamboni, R. Fischer, L. Ruesh, S. Guldimann, V. Theus, E. Schenker, and J. Renz, *Helv. Chim. Acta,* **41**, 1061 (1958).
[35]MCH-5n-cap C18 30 cm x 4 mm column; methanol:acetonitrile (20:80) eluent; 1.5 mL/min flow rate; uv detector at 254 nm.
[36]Based upon a procedure by Truce: W. E. Truce, D. P. Tate and D. N. Burdge, *J. Amer. Chem. Soc.*, 82, 2872 (1960).
[37] MCH-5n-cap C18 30 cm x 4 mm column; methanol: acetonitrile (20:80) eluent; 1 mL/min flow rate; uv detector at 254 nm.
[38]8" silica gel plate; benzene, chloroform and ethanol eluents; uv and iodine visualization.
[39]It was later found that if left in solution, decomposition would occur slowly, leading to formation of the starting material.
[40] C18 column;75/25 H_2O/MeOH mixture; 1 mL/min; 254 nm UV detector.

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